Perfusion Analysis Using Dynamic Arterial Spin Labeling (DASL)

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A variety of magnetic resonance (MR) techniques have proved useful to quantify perfusion using endogenous water as a blood flow tracer. Assuming that water is a freely diffusible tracer, the model used for these techniques predicts that the quantitation of perfusion is based on three parameters, all of which can depend on blood flow. These are the longitudinal tissue relaxation time, the transit time from point of labeling to tissue, and the difference in tissue MR signal between an appropriate control and the labeled state. To measure these three parameters in parallel, a dynamic arterial spin labeling (DASL) technique is introduced based on the analysis of the tissue response to a periodic time varying degree of arterial spin labeling, called here the labeling function (LF). The LF frequency can be modulated to over determine parameters necessary to define the system. MR schemes are proposed to measure the tissue response to different LF frequencies efficiently. Sprague-Dawley rats were studied by DASL, using various frequencies for the LF and various arterial pCO₂ levels. During data processing, the periodic behavior of the tissue response to the LF allowed for frequency filtering of periodic changes in signal intensity unrelated to perfusion and arterial spin labeling. Measures of transit time, tissue longitudinal relaxation time, and perfusion agreed well over a range of LF frequencies and with previous results. DASL shows potential for more accurately quantifying perfusion as well as measuring transit times associated with arterial spin labeling techniques. Magn Reson Med 41:299–308, 1999. © 1999 Wiley-Liss, Inc.

Key words: cerebral blood flow; MR; hypercapnia; transit time; frequency filtering

Tissue perfusion is an important physiological parameter that can be monitored non-invasively by several magnetic resonance (MR) techniques using water as an endogenous tracer (1–7). All these techniques rely on the measurement of the difference in signal intensity between a labeled state (blood spins perturbed with respect to the tissue spins) and a control state (blood spins not perturbed with respect to the tissue spins), which is proportional to perfusion. Three issues related to these techniques require further improvement. First, the signal-to-noise ratio (SNR) needs to be improved such that perfusion maps can be acquired faster and lower perfusion levels can be detected. Second, the determination of perfusion depends on a number of parameters, including the efficiency of labeling, the transit time of labeled spins to tissue, the relaxation of water in tissue, and the change in signal intensity due to labeling (3,8,9). These parameters are usually determined in separate experiments, and perfusion quantitation might be improved if all these parameters were estimated simultaneously. Finally, the model used to analyze MR perfusion experiments that use endogenous water as a perfusion tracer requires further elaboration to obtain a more detailed understanding of the physiological exchange of intravascular and extravascular water for the various size scales (carotids up to capillaries) of the vascular system (10–13). These challenging issues require continued development of perfusion measuring techniques.

Instead of measuring the tissue magnetization perfused with and without labeled arterial water, it should be possible to perform a transient or dynamic analysis of the tissue water signal. This can be accomplished by measuring the tissue signal response to a periodic arterial spin labeling function. A dynamic spin labeling approach has at least two advantages. First, the transient can be continuously repeated so it can be analyzed in detail, and second, the periodic time response is suitable for frequency filtering or correlation techniques. A dynamic analysis is perfectly adapted to the arterial spin labeling technique, where, in this case, the time varying perturbation can be the RF-driven degree of labeling. We refer to the time varying degree of labeling as the labeling function (LF) and to the dynamic arterial spin labeling approach as DASL.

In the present work, we derive the theoretical basis for analyzing regional cerebral blood flow using a square LF of arbitrary frequency. Using pulse schemes specially adapted to acquire MR data suitable for DASL, we show that perfusion, tissue longitudinal relaxation times, and transit times can be measured in parallel and are in excellent agreement with previous measurements that relied on separate experiments (8,13,14). In addition, the ease with which frequency filtering can be applied to remove periodic noise is demonstrated.

THEORY

In the following development, we will assume that macromolecules that give rise to magnetization transfer (MT) effects are saturated and the arterial blood magnetization at the labeling plane is solely determined by the arterial spin labeling procedure. A parallel theoretical development could be done without the assumption that macromolecules are saturated (8,9,15,16).

Asuming water is a freely diffusible tracer, the equation describing the time evolution of the longitudinal magnetization of water during an arterial spin labeling experiment...
is given by (8):

\[
\frac{dM_b(t)}{dt} = \frac{M_b^0 - M_b(t)}{T_{1b}} - k_{sor}M_b(t)
+ (1 - 2e^{-t/T_{1\lambda\alpha}}) \frac{f}{\lambda} M_b^0 - \frac{f}{\lambda} M_b(t),
\]

where

\( f = \) tissue perfusion rate

\( T_{1b} = \) spin-lattice relaxation time of brain water protons in the absence of perfusion and cross relaxation with macromolecules

\( T_{1a} = \) spin-lattice relaxation time of arterial water protons in the absence of cross relaxation with macromolecules

\( \tau = \) transit time of the arterial water protons from the labeling plane to the exchange site in the brain

\( M_b = \) longitudinal magnetization of water per gram of brain tissue \( M_b^0 = \) equilibrium value of \( M_b \)

\( k_{sor} = \) magnetization transfer rate constant between tissue water and macromolecules

\( \lambda = \) brain: blood partition coefficient for water

\( \alpha_0 \) = degree of labeling near the labeling plane

In the case of a time varying degree of labeling, \( \alpha_0 \) is replaced by \( \alpha(t) \), which characterizes the LF, and the integration of Eq. [1] yields

\[
M_b(t) = T_{1sat}M_b^0\left(\frac{1}{T_{1b}} + \frac{f}{\lambda} + k_{sor}e^{-t/T_{1sat}}\right)
- 2M_b^0\frac{f}{\lambda}e^{-t/T_{1\lambda\alpha}}\alpha(t) \otimes e^{-t/T_{1sat}},
\]

where

\[ \frac{1}{T_{1sat}} = \frac{1}{T_{1b}} + k_{sor} + \frac{f}{\lambda} \]

and where \( \otimes \) denotes the convolution product.

Note that the transit time of the blood protons from the labeling plane to the measuring plane, \( \tau \), not only reduces the effective degree of labeling at the exchange site, but also introduces a delay between the labeling time and the tissue response. For \( t > T_{1sat} \), a steady-state response to the LF is reached and Eq. [2] can then be written as:

\[
M_b(t) = T_{1sat}M_b^0\left(\frac{1}{T_{1b}} + \frac{f}{\lambda}\right)
- 2M_b^0\frac{f}{\lambda}e^{-t/T_{1\lambda\alpha}}\alpha(t) \otimes e^{-t/T_{1sat}}.
\]

We now define the shape of the LF. Theoretically, it can be any function of time, provided that spins can be inverted accordingly. If the LF is a square function oscillating between 0 and \( \alpha_0 \) with a period \( 2\Delta \), an analytical solution to Eq. [3] can be found for the steady state. A period of the tissue magnetization response to a square LF is given by (cf. Appendix for derivation): for \( 0 < t \leq \Delta \),

\[
\alpha(t) = \alpha_0, \quad \text{and for } \tau < t \leq \Delta + \tau,
\]

\[
M_b(t) = M_b^0T_{1sat}\left[\frac{1}{T_{1b}} + \frac{f}{\lambda} - 2e^{-t/T_{1\lambda\alpha}}\frac{f}{\lambda}\right]
\times \left\{ (1 - e^{-\Delta/T_{1sat}})e^{-t/T_{1sat}} - e^{-\Delta/T_{1sat}} + (1 - e^{-t-\Delta/T_{1sat}}) \right\}
\]

and for \( \Delta < t \leq 2\Delta \), \( \alpha(t) = 0 \), and for \( \Delta + \tau < t \leq 2\Delta + \tau \),

\[
M_b(t) = M_b^0T_{1sat}\left[\frac{1}{T_{1b}} + \frac{f}{\lambda} - 2e^{-t/T_{1\lambda\alpha}}\frac{f}{\lambda}\right]
\times \left\{ (1 - e^{-\Delta/T_{1sat}})e^{-t/T_{1sat}} - e^{-\Delta/T_{1sat}} \right\}
+ (1 - e^{-2\Delta/T_{1sat}})e^{-t-\Delta/T_{1sat}}
\]

A simulation of Eq. [4] is presented in Fig. 1 for two different LF frequencies. Figure 1 depicts three key features. First, the time at which maximum tissue magnetization is obtained is shifted with respect to the LF by a duration equal to the transit time, which is independent of the LF frequency. Second, an increase in the LF frequency leads to a decrease in the amplitude of the oscillations of the tissue magnetization. Third, the averaged tissue magnetization over a whole LF period decreases as the LF frequency increases. These changes demonstrate the fact

![FIG. 1. Simulation of the tissue magnetization response to two different frequencies of the arterial labeling function (LF) after a steady state has been reached. a: The LF frequency is 0.20 Hz. B: The LF frequency is 0.67 Hz. The dashed line shows the arterial LF (right-hand scale), and the dark line shows the response of the tissue magnetization (left-hand scale). The labeling is "off" when the degree of labeling is zero with the RF at the control plane and "on" when the degree of labeling is \( \alpha_0 \) with the RF at the labeling plane. Note for each LF cycle the delayed response of the tissue (due to the transit time, \( \tau \)), and the modulation of the amplitude of the tissue magnetization response when the LF frequency is increased. For these simulations the following parameters were used: \( T_{1sat} = 0.45 \) sec, \( \tau = 0.25 \) sec, \( f = 2 \) ml/g/min, and \( \alpha_0 = 0.8 \).]

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that the tissue magnetization is sensitive to the LF frequency in terms of phase and amplitude.

MATERIALS AND METHODS

NMR Techniques

NMR experiments were performed with a 74 mm diameter Bruker volume coil (Bruker Instruments, Billerica, MA) positioned in the center of a 4.7 T, 40 cm bore Bruker Avance-DBX spectrometer equipped with a 15 cm shielded gradient set. MR signal was acquired from a $5 \times 5 \times 4 \text{ mm}^3$ voxel using a volume-localized stimulated echo, STEAM, sequence with TE 6.3 msec and TM 8.5 msec (17). Over a bandwidth of 15 kHz, 1024 points were acquired. The voxel was positioned on the left hemisphere of the brain and included both gray and white matter. A single acquisition gave sufficient SNR so that signal averaging was not necessary. The typical spectral linewidth from the voxel was 60 Hz. To avoid the influence of vascular spins in the experiment, crusher gradients of 5.2 G/cm and 2 msec duration, one or many LF periods can be added to the TR interval and the transient can be sampled at a defined phase of its evolution with respect to the LF, as depicted in Fig. 2b. This allows for the use of long TRs while maintaining a steady-state response to the LF and obtaining a high temporal resolution. We refer to this scheme as interleaved dynamic acquisition. Stated another way, in the interleaved dynamic acquisition scheme, the LF and the acquisition are performed at two different frequencies, such that, due to their frequency offset, each acquisition occurs at a different phase of the LF cycle. To implement the inter-

Measurement of the Optimized Control Frequency for Arterial Spin Labeling

Since MT effects arise during the labeling, it is necessary to devise a scheme in which macromolecules are perturbed equally with and without labeling. Under the assumption that the frequency dependence of the MT effects was symmetric around the water frequency, Zhang et al (8) obtained a control spectrum by simply switching the sign of the labeling frequency. However, Pekar et al have recently reported a small but detectable asymmetry of MT effects (18). To control accurately for off-resonance MT effects we optimized our control frequency in the following way. Eighty spectra were acquired for each rat, separated by either a 2 sec "labeling" or a 2 sec "control" radiofrequency (RF) pulse during which no gradient was applied such that the measured signal was dependent on MT effects but not on perfusion. Forty spectra were acquired after a "labeling" RF pulse and 40 after a "control" RF pulse, in an interleaved fashion (5 label then 5 control) x 8 loops). This experiment was repeated for four different control frequencies (8000, 8250, 8500, and 9000 Hz) while maintaining the labeling frequency constant at $-8500 \text{ Hz}$. The difference between "control" and "label" tissue magnetization as a function of the control frequency was fitted to a straight line, and the optimized control frequency was derived for each rat as being the one giving a theoretical null difference between spectra acquired after a "control" RF pulse and spectra acquired after a "labeling" RF pulse. This optimized control frequency was thereafter used in all subsequent experiments for each animal. For all seven animals used in this study, the optimized control frequency was significantly shifted to lower frequencies by $280 \pm 106 \text{ Hz}$ relative to the expected symmetric control frequency.

Dynamic Perfusion MR Acquisition Schemes

The fastest method to sample the transient tissue magnetization response to the LF consists of interleaving short arterial labeling or control RF pulses with data acquisition. This direct dynamic acquisition scheme is presented in Fig. 2a. The temporal resolution at which the transient tissue response can be sampled by the direct dynamic acquisition scheme is limited by the SNR. The higher the temporal resolution, the lower the SNR will be.

To maintain both SNR and temporal resolution, one can take advantage of the fact that the labeling scheme is periodic. Instead of acquiring the data after a short labeling duration, one or many LF periods can be added to the TR interval and the transient can be sampled at a defined phase of its evolution with respect to the LF, as depicted in Fig. 2b. This allows for the use of long TRs while maintaining a steady-state response to the LF and obtaining a high temporal resolution. We refer to this scheme as interleaved dynamic acquisition. Stated another way, in the interleaved dynamic acquisition scheme, the LF and the acquisition are performed at two different frequencies, such that, due to their frequency offset, each acquisition occurs at a different phase of the LF cycle. To implement the inter-

FIG. 2. Possible MR acquisition schemes to sample the tissue response to dynamic arterial spin labeling. a: The direct dynamic acquisition scheme. The duration of the labeling RF (on) and the control RF (off) is $P$. In this case, $TR$ is much shorter than the period of the labeling function (LF). After each acquisition, the duration of recovery is short such that low acquisition pulse tip angles may be required. Therefore, the SNR decreases as the temporal resolution in sampling the transient increases. However, the transient is sampled as fast as it occurs. b: The interleaved dynamic acquisition scheme. On this example, $TR$ is longer than the LF period so that the acquisition occurs at different phases of the periodic tissue response. The fact that the labeling scheme is segmented into short pulses of duration $P$ enables acquisition at any phase in the tissue magnetization evolution. After each acquisition, the duration for recovery is not limited by the temporal resolution in sampling the transient. Low tip angles can be avoided to maximize the SNR. For both schemes, the tissue magnetization is assumed to have reached a steady state. The labeling RF time course, the signal acquisition time course, and the tissue magnetization time course are all plotted on the same time scale. The echoes on the signal acquisition time course and the crosses on the tissue magnetization time course represent signal acquisitions.
leaved dynamic acquisition scheme, the LF was fragmented into a train of RF pulses of duration P (typically 200 msec), separated by a very short delay (typically 100 msec). A square LF of period 2Δ was obtained by applying n pulses at the labeling frequency followed by n pulses at the control frequency such that

\[ \Delta = nP. \]  

Segmentation of the labeling pulse creates multiple windows for MR signal acquisition, allowing TR to vary independently of the LF period. A noteworthy case occurs for:

\[ TR = 2m\Delta + P + \text{Acq}, \]  

where m is any positive integer and Acq is the duration of the acquisition sequence. For this case, each signal acquisition is shifted from the LF cycle by a duration P. At the end of the experiment, the data set looks like it was acquired every \( P \) instead of every TR. No data re-ordering is necessary when Eq. [7] is fulfilled. However, note that TR can more generally be \( mP + \text{Acq} \), which can correspond to the acquisition of more than one point per cycle of LF. In this more general case, data reordering is required such that the data appears as if it were acquired every \( P \). Notably that \( TR = P + \text{Acq} \) yields the previously described direct dynamic acquisition scheme.

Fourier Transform of the Tissue Response

Because the labeling scheme and the tissue response are periodic, the Fourier transform (FT) of the data is very useful to visualize frequency components due to the tissue response to the LF as well as to periodic noise due to physiological fluctuations or instrumental instabilities. To analyze the data in the frequency domain, it is important to note that the interleaved dynamic acquisition scheme described above is related to two distinct frequency scales: the acquisition frequency scale, between -1/2TR and 1/2TR, based on the effective duration separating two acquisitions, and a phase frequency scale, between -1/2P and 1/2P, that represents the dephasing of the LF cycle relative to the acquisition cycle. Both physiological and instrumental noise may be dependent or independent of the LF cycle; however, noise due to both of these sources will appear in the FT of the data.

Measurement of the LF in the Carotid

To determine whether the blood magnetization was following the applied RF, the LF was measured on two separate rats. The rats were prepared as described above, but positioned to observe a region 1 cm posterior, such that the junction between the spine and the brain was in the center of the magnet. A STEAM voxel \((3 \times 3 \times 4 \text{ mm}^3)\) was centered over one of the carotid arteries. Other acquisition parameters were the same as previously described, except the labeling frequency was at -4250 Hz and the control frequency was at +4250 Hz, so that the labeling plane would be at the same location as during the DASL experiments performed in the brain. We also assumed that the MT effects in circulating blood were small enough that MT asymmetry was negligible (19). The LF frequency was 0.62 Hz and 40 spectra were acquired per period of LF.

Data Processing

All the data processing was performed automatically using Matlab 5 (The MathWorks, Natick, MA) on a Sun Workstation (Sun Microsystems). All the fits were performed with a Nelder-Mead type simplex search method (function “fmins”). After FT and magnitude calculations, all the spectra belonging to a single scan were displayed superimposed on each other, and acquisition consistency was visually examined.

After peak integration (between -200 Hz to +200 Hz), data from the dynamic perfusion acquisition scheme were submitted to a baseline correction algorithm. The baseline was derived by replacing each LF period by its average (mean time and mean intensity). The set of points so obtained was fitted to a polynomial whose order was a function of the number of points in the set. That polynomial, without its zero order term, was used to perform a point by point subtraction, leading to a baseline correction.

Data were then fitted to Eq. [4] using the previously determined values of \( T_{1b} = 1.6 \text{ s}, T_{1b} = 1.5 \text{ s}, \lambda = 0.9 \text{ ml/g, and } \alpha = 0.8 \) (8). Frequency filtering was performed as follows: the FT of the fit and the FT of the data were computed. Using a threshold level (\(<1\% \) of the largest peak), a complex filter (two arrays of 0 and 1, one for the real part and one for the imaginary part) was derived from the real and from the imaginary components of the FT of the fit. This filter was then applied to the FT of the data by performing point by point multiplications between the components of the filter and the components of the FT data. The data were back-transformed to the time domain. Filtered data were then fit to Eq. [4] a second time to improve the precision of the fit. \( M_{0b}^r, T_{1sat}, \tau, \) and f were the parameters obtained from the fit.

As a consistency check, we verified that Eq. [4] was stable to filtering. An ideal set of data was generated using Eq. [4]. This simulated data set was Fourier transformed, filtered, back transformed, and fitted with Eq. [4] to check whether the result yielded the original parameter values used to generate the ideal set of data. Furthermore, generating multiple sets of ideal data using the same LF frequency but various parameter sets \((M_{0b}^r, T_{1sat}, \tau, \) and f\) showed that the peaks in the FT of the data had a stable position but a varying amplitude. Based on the position of the peaks, our filter did not influence the estimation of the parameters in a systematic way.

Rat Preparation

Seven male Sprague-Dawley rats (200–300 g, Taconic, Germantown, NY) were initially anesthetized with 5% halothane, intubated, and then maintained on 0.8% halothane in a 1.25:0.75 O2:N2 mixture using a rodent ventilator (Harvard Apparatus, South Natick, MA). The ventilator was set between 78 and 85 strokes/min with a volume of 2.0–2.5 cm3 to maintain baseline arterial pCO2 between 30 and 50 mmHg. A line was placed in the femoral artery for blood pressure monitoring and for blood gas analysis (ABL50, Radiometer, Copenhagen, Denmark). A dose of 0.6 ml of pancuronium bromide (Oragon, W. Orange, NJ) was
administered intraperitoneally every hour to maintain immobilization of the rats. Animals were placed on a thermostated water pad receiving a continuous flow of heated water to maintain body temperature. The labeling plane did not cross the path of the water circulation. The rat’s body was also covered with a gauze blanket to achieve maximum temperature stability. A rectal probe was used to monitor and maintain the body temperature at $37.7^\circ C$.

Head movements were minimized by using a bite bar, ear fixations, and tape on the neck and on the nose.

**Experimental Protocol**

Data were acquired as follows, for each of the seven rats:

1. The optimized control frequency was determined for subsequent use in the entire experiment.
2. A blood sample was collected for blood gas analysis.
3. Dynamic perfusion measurements were obtained for five different LF frequencies, using the interleaved dynamic acquisition scheme. $P = 200$ msec. Note that Eq. [7] is always fulfilled:
   - $2\Delta = 1.6$ sec (0.62 Hz), $TR = 3.4$ sec, 8 LF periods were sampled.
   - $2\Delta = 2.0$ sec (0.50 Hz), $TR = 4.2$ sec, 4 LF periods were sampled.
   - $2\Delta = 3.2$ sec (0.31 Hz), $TR = 3.4$ sec, 4 LF periods were sampled.
   - $2\Delta = 4.0$ sec (0.25 Hz), $TR = 4.2$ sec, 3 LF periods were sampled.
   - $2\Delta = 6.0$ sec (0.17 Hz), $TR = 6.2$ sec, 2 LF periods were sampled.

Note in all cases that $TR$ was greater than $5T_{1sat}$ ($T_{1sat}$ was approximately 500 msec, data not shown).

4. Another blood sample was collected for blood gas analysis.
5. Inspired $CO_2$ was increased, leading to a change in arterial $pCO_2$ level. After a 10–15 min delay to reach steady state, steps 2–5 were repeated.

A total of three inspired $CO_2$ levels were used: no added $CO_2$, 6% $CO_2$, and 12% $CO_2$ in the inspiration gases for each rat. The total experiment duration was 4 hr, during which six blood samples (less than 0.2 ml each) were collected.

**RESULTS**

Figure 3 shows data obtained from a voxel centered over the carotid artery, using the direct dynamic acquisition scheme without filtering. Spectra were acquired every 40 msec, demonstrating that the blood magnetization followed the RF-driven LF quite well. The effect of blood pulsation introduces a small noise contribution that can be seen as oscillations on the experimental LF. The value of $\alpha_0$ in the carotid was not derived because the voxel also included some tissue. For all fitting, $\alpha_0$ was assumed to be 0.8, as previously measured (8).

Figure 4 shows data obtained with the interleaved dynamic scheme and three different LF frequencies, with the best fit to these data prior to performing any frequency filtering. Amplitude modulation was clearly visible as the LF frequency increased. The time scale displayed represents the one related to the transient tissue response (as if data were acquired every $P$) and not to the acquisition scheme (data are acquired every $TR$).

Figure 5 demonstrates the process of frequency filtering for one set of experimental data. Data and the best fit to Eq. [4] are shown in Fig. 5a. The real and imaginary components of the FT of both the data and the fit are shown in Fig. 5b. The filter is based on the intensity of the frequency components of the FT of the fit. Figure 5c shows the frequency components left in the FT of the data after filtering. Finally, in Fig. 5d, the back FT of the data is shown with the new best fit to Eq. [4]. For this case, a frequency component at 0.037 Hz on the $TR$-related frequency scale is present in the data but is not fit by the model (Fig. 5b, real and imaginary parts). The position of this peak was dependent on the $TR$ value but not on the LF frequency (data not shown), indicating that this peak had no relation to the oscillating behavior of the LF. Interest-
ingly, $1/0.037 \text{ Hz} \approx 27 \text{ sec}$ is an integer multiple of the respiratory period (0.75 sec) indicating that this periodic fluctuation was due to respiratory motion. This signal fluctuation and other fluctuations at frequencies not predicted by the model are readily removed, as illustrated in Fig. 5d. The efficiency of the frequency filtering can be estimated by comparing the value of $\chi^2$ before and after filtering. The averaged $\chi^2$ over five LF frequencies and three arterial pCO$_2$ levels obtained on one rat that we studied was $21.2 \pm 7.7$ before filtering and after filtering $\chi^2$ dropped to $2.7 \pm 3.6$, indicating a much improved fit.

DASL data from five LF frequencies and three different arterial pCO$_2$ levels from all the rats were fitted to Eq. [4]. The derived values of $T_{\text{sat}}$, $\tau$, and $f$ are presented in Fig. 6, as a function of arterial pCO$_2$, for five different LF frequencies. The trends in the parameters are in good agreement with previous measurements (8,20). Fig. 6a shows $1/T_{\text{sat}}$ and it is clearly visible that, at the lowest LF frequency, $1/T_{\text{sat}}$ increases slightly with arterial pCO$_2$. This is predicted by the perfusion model (Eq. [1]) and has been previously observed in the heart by Williams et al (20). Indeed, the fact that $T_1$ is dependent on blood flow is the basis for flow-sensitive alternating inversion recovery (FAIR)-type perfusion schemes (3,6,7). $\tau$ values at normal arterial pCO$_2$ (Fig. 6b) are in good agreement with ones previously measured by H. Nakagawa et al using $^{51}$Cr-labeled red cells (21) and by Zhang et al using arterial spin labeling (8). $\tau$ decreases as arterial pCO$_2$ increases as expected. Finally, the derived values of $f$ (Fig. 6c) show a large increase with arterial pCO$_2$, as previously reported. The quantitative perfusion values obtained have been corrected for the influence of the transit time as measured with DASL.

To assess whether changing the LF frequency altered the estimation of $T_{\text{sat}}$, $\tau$, and $f$, the values obtained at each LF frequency were compared. For each arterial pCO$_2$ level, the parameter values derived from the four highest LF frequencies were compared with the values derived from the lowest LF frequency used. The lowest LF frequency was equivalent to the steady-state case (8). The mean relative
The differences and their standard deviations, for the low and high arterial pCO2 levels, are shown in Fig. 7 as a function of the LF frequency. For $T_{1sat}$ and $\tau$, all the differences were insignificant ($P < 0.05$), except for one. The estimation of $T_{1sat}$ derived from the highest LF frequency differs significantly at high pCO2 compared with the value derived at the lowest LF frequency. The three highest LF frequencies yielded an estimation of $f$ that differed slightly from the estimation at the lowest LF frequency. It can be seen in Fig. 7c that perfusion increases as the LF frequency decreases. The highest and the lowest LF frequencies yield perfusion values that differ by 10%. This small variation is probably due to a systematic error. Data were acquired sequentially from the highest to the lowest LF frequency. Perfusion probably increased during the time it took for the acquisition of all five LF frequencies. The arterial pCO2 measurements made in the blood samples tended to increase with time (data not shown). Future comparisons should randomize different LF frequency order.

Figure 8 shows the variations of $\tau$ as a function of $f$. As expected, $\tau$ decreases as $f$ increases. Interestingly, at high values of $f$, blood flow can increase without an additional decrease in $\tau$. Similar behavior has been previously observed by Grubb et al using the non-diffusive radioactive tracer, C15O-hemoglobin, in the monkey brain (22).

**DISCUSSION**

In this study, we show that using DASL yields a parallel measure of $T_{1sat}$, $\tau$, and $f$. We also show that the tissue response can be efficiently measured using periodic excitation and periodic acquisition schemes. The LF, monitored in the carotid artery, followed the input RF function quite well. It was necessary to take account of the asymmetry of the MT effects to obtain accurate data. Data at five different LF frequencies and at fairly constant arterial pCO2 levels yielded estimations of $T_{1sat}$, $\tau$, and $f$ that were in good agreement with each other. The tissue response was in agreement with the theoretical predictions over the range of five LF frequencies that we used ($1/12T_{1sat}$ to $1/3T_{1sat}$). Finally, it was demonstrated that DASL readily lends itself to frequency filtering for removing various types of periodic noise. In this study STEAM was used to acquire data for detailed analysis; however, it should be straightforward to replace STEAM with a fast imaging technique to obtain maps of perfusion and transit time.

Variations of $\tau$ with changes in $f$ have been previously reported (22). In the rat brain, it was demonstrated that an increase of the arterial pCO2 level from 40 to 80 mmHg
leads to a reduction of $\tau$ from 300 msec to 100 msec. Therefore the effective degree of arterial labeling at the brain region sampled increases from 0.66 to 0.75, assuming that $\alpha_0 = 0.8$ at the point of labeling, and $T_{1b} = 1.5$ sec. Without this correction, $f$ can be overestimated at higher flows or underestimated at lower flows, because the effective degree of labeling at the exchange site is directly related to $\tau$. The fact that DASL simultaneously measures $\tau$ and $f$ enables a more accurate quantitation of perfusion to be derived. This might be useful for activation studies since changes in blood velocities in the large vasculature of the brain during task activation have been previously reported (23).

Frequency filtering of data obtained by DASL can suppress periodic perturbations, even those occurring at low frequencies, due to the predicted shape of the frequency spectrum for the tissue response (Fig. 5b). Filtering and fitting also suppresses any signal whose frequency response differs from the one predicted by the model. Clearly, signal fluctuating at the respiratory and heart rates can be distinguished from changes due to arterial spin labeling if the LF is chosen properly. The LF has to be chosen to make sure that no periodic perturbation will fold over into a frequency component due to perfusion. Alternatively, a physiological parameter can be used to control the LF frequency. For example, the LF frequency could be chosen such that the labeling would occur when blood is at its maximum velocity through the carotid during systole, thus maximizing the arterial degree of labeling in the tissue and potentially minimizing RF power deposition.

It should be possible to eliminate large vessel contributions to perfusion images using DASL. Assuming, first, that a good definition of perfusion is given by Eq. [1], and that water protons in large vessels do not exchange with tissue and therefore do not behave as predicted by Eq. [1], one can reasonably assume that the water protons present in larger vasculature will have a dynamic response different from that of the water protons exchanging with tissue and so can be filtered out from the final fit for perfusion. Ye et al have previously noticed differences during the wash-in of the labeled water when signal from a larger vessel is present and when it has been destroyed using crusher gradients (13). Using a frequency filtering technique or a correlation technique, the perfusion signal may be separated from signal arising from vessels (or vice versa for angiography).

Frequency filtering would be more easily implemented with DASL using a sinusoidal LF. Because the frequency spectrum of the tissue response would be simpler than the one obtained with the square LF, filtering would be easier. Fitting would also be easier because the sine function is continuous as are its first derivative versus time and versus all the fitted parameters. Fitting would also be more accurate since the regular sampling we propose is more suitable for a sinusoidal function than for an exponential function. In the case of a square LF, since the head and the tail of the exponential decay are equally sampled, the end of the exponential is oversampled, introducing a potential for a small, systematic underestimation of $T_{1sat}$. The sinusoidal tissue response could be sampled based on the Nyquist criteria (2 points per period of LF), allowing for a complete characterization of the tissue through its response to a large range of LF frequencies very rapidly. Finally, the transit time could be derived from the estimation of a phase shift, as the time at which the maximum signal is obtained during an LF cycle, provided that at least two LF frequencies are studied. A sinusoidal and a square LF are certainly not the only shapes possible. More complex LFs could be designed that may have advantages to characterize blood flow further. For example, the LF could be the sum of three sine waves at three different frequencies. The FT of the tissue response should enable separation of the three tissue responses at the three different frequencies contained in the LF. In this way, $T_{1sat}$, $\tau$, and $f$ could be measured with a single DASL acquisition.

Five LF frequencies were studied to examine the tissue response over a wide range of perturbations. One frequency of the LF is sufficient to fit to Eq. [4]. Based on the tissue response to five different frequencies of a square LF and deriving for each LF frequency the value of $T_{1sat}$, $\tau$, and $f$, it appears that a good compromise between SNR and response to the LF is obtained when using a frequency for the square LF of (1/6)$T_{1sat}$ (This situation corresponds to our data acquired with an LF at 0.31 Hz). At this particular frequency, the wash-in and the wash-out are both sampled during $3T_{1sat}$, which is long enough to make an accurate determination of $T_{1sat}$. After $3T_{1sat}$ of wash-in, 95% of the full amplitude of the tissue response is obtained, which is large enough to determine perfusion accurately.

The acquisition scheme used was sensitive to MT, because the MT asymmetry was readily detected and correction was necessary to obtain accurate measurements. Indeed, unlike perfusion-induced changes of the tissue magnetization, MT effects occur without any transit time delay after the labeling frequency is switched to the control frequency or vice versa. Therefore, any asymmetry would mask the effect of transit time and would introduce an error in the estimation of $\tau$. Moreover, an asymmetry would modify the amplitude of the tissue response to arterial labeling and introduce an error in the estimation of perfusion as well. For the present work, shifting the control frequency was easier and less time consuming than the rigorous correction for MT asymmetry proposed by J. Pekar et al (18). Note, however, that the asymmetry measured here is in good agreement with the one Pekar et al reported. The approach of determining the proper control frequency to compensate for the MT asymmetry can be applied in imaging techniques, because Pekar et al reported that the asymmetry was comparable for gray matter and for white matter. New labeling schemes that better control for MT (24) or the use of two coil approaches to avoid MT (15,16) will minimize this problem.

A drawback of using a square LF is that as the LF frequency increases, the time interval on which the $T_{1sat}$ estimation is based (half the duration of the LF period) decreases, leading to larger errors in the estimation of $T_{1sat}$. There is a simple solution to improve the measurement, in the case of a single coil approach for labeling. By measuring the evolution of the magnetization from equilibrium to the steady state one can obtain a measurement of $T_{1sat}$ and $k_{for}$ with a greater precision than the one obtained here. Moreover, since $T_{1sat}$ and $k_{for}$ would be known, no assumption would be necessary for the value of $T_{1sat}$ and $k_{for}$, and it would reduce the fitted parameters in Eq. [4] to $f$ and $\tau$. ...
An interesting physiological relation that we measured with DASL was the one between the transit time of arterial water protons and regional blood flow. With DASL, both parameters were determined simultaneously. A clear decay can be observed in $\tau$ when $f$ starts increasing. Interestingly, at higher values of $f$, $\tau$ plateaus. If one assumes that between the labeling plane and the tissue exchange site, there is no significant loss of labeled water then the $\tau$ value we measured may be of interest. The transit time does not represent the duration that the tracer spends in the tissue and therefore, the central volume principle cannot be applied here to derive the tissue blood volume. However, some considerations regarding the blood volume can be made based on the data. A one to one relation between the transit time from labeling plane to tissue and the tissue perfusion would imply that the blood volume between the labeling plane and the tissue remains constant and the blood flow is varying. However, if transit time does not increase with tissue perfusion, as seen at high values of arterial $pCO_2$, then this implies that the blood volume is increasing to provide the required blood supply. Therefore, alteration in the relation between $\tau$ and $f$ should be highly sensitive to changes in blood volume and may be very useful for characterization of pathophysiological conditions.

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**APPENDIX: ANALYTICAL INTEGRATION OF EQ. [3] AT STEADY STATE**

Eq. [1] can be rewritten as:

$$\frac{dM_b(t)}{dt} = M_b^0 \left( \frac{1}{T_{b1}} + \frac{f}{\lambda} \right) - \frac{1}{T_{sat}} M_b(t) - 2 M_b^0 \frac{f}{\lambda} \alpha(t)e^{-\gamma/T_s} \quad \text{[A1]}$$

In that equation, the term $T_{sat}M_b^0(1/T_{b1} + f/\lambda)$ can be considered as a baseline offset and therefore does not need to be carried through the following analysis. We do not consider the effects of the transit time either (degree of labeling in the tissue smaller than $\alpha$ and delayed tissue response), since it has no relationship with the modulation in the case of a square LF. We now define

$$A = 2 \frac{\alpha_0 f}{\lambda} M_b^0.$$  

We consider that $\tilde{M}_b(0)$ is the value of the magnetization, macromolecules being fully saturated (control state). Switching labeling “on” at $t = 0$ ($\alpha(t) = 0 \rightarrow \alpha(t) = \alpha_0$) yields:

$$\tilde{M}_b(t) = \tilde{M}_b(0)e^{-\gamma/T_{sat}} - AT_{sat}(1 - e^{-\gamma/T_{sat}}). \quad \text{[A2]}$$

Switching the labeling “off” at $t = \Delta$ yields:

$$\tilde{M}_b(t) = [\tilde{M}_b(0)e^{-\gamma/T_{sat}} - AT_{sat}(1 - e^{-\gamma/T_{sat}})]e^{-\beta(\Delta-T_{sat})}. \quad \text{[A3]}$$

The steady state of the oscillations of the tissue magnetization is described by:

$$\tilde{M}_b(0) = \tilde{M}_b(2\Delta) \quad \text{[A4]}$$

$$\tilde{M}_b(0) = [\tilde{M}_b(0)e^{-\gamma/T_{sat}} - AT_{sat}(1 - e^{-\gamma/T_{sat}})]e^{-\gamma/T_{sat}}. \quad \text{[A5]}$$

We define and obtain:

$$e^a \tilde{M}_b(0) = \tilde{M}_b(0)e^{-\beta} - AT_{sat}(1 - e^{-\beta}) \quad \text{[A6]}$$

$$(e^a - e^{-\beta})\tilde{M}_b(0) = AT_{sat}(e^{-\beta} - 1) \quad \text{[A7]}$$

$$\tilde{M}_b(0) = \frac{AT_{sat}(e^{-\beta} - 1)}{e^a - e^{-\beta}} \quad \text{[A8]}$$

$$= 2M_b^0 T_{sat}\alpha_0 \frac{f}{\lambda} \left( \frac{e^{-\gamma/T_{sat}} - 1}{e^{\gamma/T_{sat}} - e^{-\gamma/T_{sat}}} \right). \quad \text{[A9]}$$

Considering now the baseline term, the control magnetization level under a steady-state periodic labeling is therefore:

$$M_b(0) = M_b^0 T_{sat}\left[ \frac{1}{T_{b1}} + \frac{f}{\lambda} \right] - 2\alpha_0 f \left( \frac{1 - e^{-\gamma/T_{sat}}}{e^{\gamma/T_{sat}} - e^{-\gamma/T_{sat}}} \right). \quad \text{[A10]}$$

Note that

$$\lim_{\Delta \to 0} M_b(0) = M_b^0 T_{sat}\left( \frac{1}{T_{b1}} + \frac{f}{\lambda} \right),$$

which is in agreement with previous theoretical developments. The amplitude of the oscillations is given by:

$$M_b^0 T_{sat}2\alpha_0 \frac{f}{\lambda} \left( 1 - e^{-\gamma/T_{sat}} \right). \quad \text{[A10]}$$

Using Eqs. [A2], [A3], and [A9], and introducing the effect of transit time, we can now write the complete solution to Eq. [A1]. The result is Eq. [4a]: for $0 < t < 1$, $\alpha(t) = \alpha_0$, and for $t > 1$, $\alpha(t) = \alpha_0 + \alpha$, and for $t > \Delta + \tau$,

$$M_b(t) = M_b^0 T_{sat}\left[ \frac{1}{T_{b1}} + \frac{f}{\lambda} \right] - 2e^{-\gamma/T_{sat}}\alpha_0 \frac{f}{\lambda} \left( \frac{1 - e^{-\gamma/T_{sat}}}{e^{\gamma/T_{sat}} - e^{-\gamma/T_{sat}}} + (1 - e^{-a(\Delta+\tau)}) \right) \quad \text{[4a]}$$
and for $\Delta < t \leq 2\Delta$, $a(t) = 0$, and for $\Delta + \tau < t \leq 2\Delta + \tau$,

$$M_0(t) = M_0^{\text{sat}} \left( \frac{1}{T_{1b}} + \frac{f}{\lambda} \right) - 2e^{-t/T_{1\text{sat}}} \frac{f}{\lambda} \left( 1 - e^{-\Delta/T_{1\text{sat}}} \right) \left( e^{-\Delta/T_{1\text{sat}}} - e^{-t/T_{1\text{sat}}} \right) + \left( 1 - e^{-t/T_{1\text{sat}}} \right) e^{-t/(\Delta + \tau - \Delta T_{1\text{sat}})} \right)$$

\[4b\]

REFERENCES