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Magnetization Transfer (MT) Enhanced Vascular-space-occupancy (MT-VASO) fMRI

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Abstract

Vascular-space-occupancy (VASO) MRI is a novel technique that employs blood signal nulling to detect blood volume alterations through changes in tissue signal. VASO has relatively low signal to noise ratio (SNR) since only 10–20% of tissue signal remain at the time of blood nulling. Here, it is shown that by adding a magnetization transfer (MT) pre-pulse it is possible to increase SNR either by attenuating the initial tissue magnetization when the MT pulse is placed before inversion, or, accelerating the recovery process when the pulse is applied after the inversion. To test whether the MT pulse would affect the blood nulling time in VASO, MT effects in blood were measured both *ex vivo* in a bovine blood phantom and *in vivo* in human brain. Such effects were found to be sufficiently small (< 2.5%) under a saturation power $\leq 3 \mu T$, length = 500 ms, and frequency offset ≥ 40 ppm to allow use of the same nulling time. Subsequently, functional MRI experiments using MT-VASO were performed in human visual cortex at 3T. The relative signal changes in MT-VASO were of the same magnitude as in VASO, while the contrast to noise ratio (CNR) was enhanced by $44 \pm 12\%$ and $36 \pm 11\%$ respectively. Therefore, MT-VASO should provide a means for increasing inherently low CNR in VASO experiments while preserving the CBV sensitivity.

Keywords

cerebral blood volume; VASO; magnetization transfer; blood; inversion recovery; SNR; CNR; fMRI; MRI

Introduction

Microvascular cerebral blood volume (CBV) is an important physiological parameter that changes during neuronal activation (1–3), and in pathologies such as ischemia and cancer (4,5). Vascular-space-occupancy (VASO) MRI (6) is a novel technique that is sensitized to such CBV alterations by acquiring images when blood signal is expected to be nulled, yet extravascular tissue signal is positive. This is achieved by applying a spatially non-selective inversion pulse followed by a waiting time (inversion time, TI) at which the longitudinal magnetization of blood is negligible. VASO was initially proposed as a functional MRI (fMRI) method because of improved spatial localization (6,7) of the neuronal activation site as compared to BOLD fMRI and has been used for several functional studies (8–12). When used for clinical applications, another advantage of VASO is that no injection of paramagnetic contrast agent is needed during the examination (4,5).

VASO MRI uses the fact that the longitudinal relaxation time (T_1) of brain tissue is shorter than that of blood. However, the use of a non-selective inversion to accomplish blood nulling leads to low tissue signal to noise ratio (SNR). At 3 Tesla (3T), depending on the repetition time (TR) used, only 10–20% of tissue signal is available and this residual tissue signal will be even lower at higher magnetic fields where the T_1 difference between blood and tissue reduces (7). Some variant VASO approaches that acquire images at inversion times where blood is not nulled can yield higher SNR (7,13), but they suffer from less straightforward interpretation and potential intravascular BOLD contamination when echo times (TE) cannot be sufficiently short, as may be the case in single-shot acquisitions.

Magnetization transfer (MT) imaging (14) employs the transfer of saturation from semi-solid proton pools to bulk water protons (14) and has been used to reduce tissue signal and thereby to enhance blood signal, such as in magnetic resonance angiography (MRA) (15). While there are large MT effects in most brain tissues, it has been reported that blood has little MT effect due to low concentration of macromolecules (14,16). A few studies have demonstrated that in inversion recovery (IR) experiments, tissue with sufficient MT effect recovers much faster when an MT pre-pulse is applied (17–20). In this paper we investigate two MT approaches that accelerate the longitudinal inversion recovery process of brain tissue in the VASO approach without affecting the blood nulling time, with the goal of increasing SNR and contrast to noise ratio (CNR).

Materials and Methods

Pulse sequences and Theory

Two MT-VASO sequences were designed as illustrated schematically in Fig. 1. In sequence I, an off-resonance MT pre-saturation radiofrequency (RF) pulse is added immediately before the VASO inversion pulse. Assuming little MT effect in blood, only the tissue signal is suppressed due to magnetization transfer. Therefore, after inversion, the tissue longitudinal recovery starts from a point closer to zero than in the original VASO and, at the same TI for blood nulling, the tissue signal will be larger than without MT preparation. In sequence II an off-resonance MT pre-saturation RF pulse is added immediately after the VASO inversion pulse leading to faster tissue recovery (17–20) and a greater signal at the same blood nulling time.

Simulations were performed to verify theoretically the behavior of tissue magnetization in these two sequences. A two-pool quantitative MT model (21,22) was employed and numerical evaluation of six coupled Bloch equations [1–6] was carried out. The resulting magnetization model is:

$$\frac{dM_z^i}{dt} = \frac{M_0^i - M_z^i}{T_1^i} - R_{ij} \cdot M_z^i + R_{ji} \cdot M_z^j + \omega_1 \cdot M_y^i \quad [1,2]$$

$$\frac{dM_x^i}{dt} = \frac{-M_x^i}{T_2^i} - 2\pi \cdot \Delta \cdot M_y^i \quad [3,4]$$

$$\frac{dM_y^i}{dt} = \frac{-M_y^i}{T_2^i} + 2\pi \cdot \Delta \cdot M_x^i - \omega_1 \cdot M_z^i \quad [5,6]$$

in which M^i is the proton magnetization of the bulk water (w) or macromolecule (m) pools, respectively, that is exchanging with the other pool (j). So for Eq. [1], $i = m$ and $j = w$, while $i = w$ and $j = m$ for eq. [2]. $R_{ij} = R \cdot M_0^j$ is the population based exchange rate between these two pools. ω_1 is the angular frequency of precession induced by the MT saturation pulse. Δ is the frequency offset of the MT pre-pulse. T_1^i and T_2^i are the longitudinal and transverse relaxation times, respectively. Typical parameter values reported in the literature (22–24) for gray matter (GM) in healthy human brain *in vivo* at 3T were used ($T_1^w = 1122$ ms, $T_1^m = 300$ ms, $T_2^w = 79$ ms, $T_2^m = 9.1 \mu s$, $M_0^w = 1$, $M_0^m = 0.05$, $R_{mw} = 40 \text{ s}^{-1}$, $R_{wm} = 2 \text{ s}^{-1}$). All computing programs were coded in Matlab 6.0 (Mathworks, Natick, MA, USA).

MRI Experiments

The studies were performed on a 3.0 Tesla MRI scanner (Philips Medical Systems, Best, The Netherlands) using the body coil for RF transmission and an 8-channel head coil for reception. The body coil is about 650 mm in axial length and spatially non-selective inversion RF pulses were used to minimize inflow effects in the VASO experiments. For this coil length, negligible inflow was found for TR-values of 5 s or longer (25). A continuous wave (CW) RF irradiation scheme was adopted to induce MT effects. Imaging parameters were: repetition time (TR) = 7 s, echo time (TE) = 4.9 ms, flip angle (FA) of excitation = 90, SENSE-factor = 2, TI for VASO = 1106 ms. For blood phantom measurements (see next sub-section), voxel volume = $1 \times 1 \times 5 \text{ mm}^3$, field of view (FOV) = $64 \times 64 \text{ mm}^2$, effective receiver bandwidth = 540 Hz/pixel, imaging matrix = 64×64 , and reconstruction matrix = 128×128 , were used. For brain, voxel volume = $2 \times 2 \times 5 \text{ mm}^3$, FOV = $210 \times 210 \text{ mm}^2$, effective receiver bandwidth = 523 Hz/pixel, imaging matrix = 105×105 , reconstruction matrix = 256×256 for better visualization. VASO fMRI is typically performed using single-shot gradient echo (GE) echo planar imaging (EPI) sequence (6,26). However this approach suffers from fat shift artifacts in the images and from BOLD contamination to the VASO contrast (10). It has been demonstrated by Poser et al. (10) that turbo spin echo (TSE) imaging can reduce both artifacts and improve the quality of VASO images. Therefore a single-shot TSE imaging sequence was employed in this study. The phase encoding order is from lower frequency to higher frequency k-space lines to achieve shortest effective TE for the low- frequency k-space lines. For brain imaging, each TSE shot consists of 53 refocusing pulses of 180° and the total readout duration is 265 ms. A ultra-short acquisition window was used to further reduce the effective TE and echo spacing (4.9 ms) in order to minimize the BOLD contamination. Higher-order shimming was employed.

Measuring the MT Effect in Blood

The main assumption in the proposed MT-VASO method is that the MT effect in blood is sufficiently small (14,16) that the blood nulling inversion time is not affected, when using moderate RF power irradiation ($2\text{--}4\ \mu T$) at frequency offsets far ($> 20\text{--}40$ ppm) from the water resonance. To verify this, the MT effect in blood was measured both *ex vivo* in a home-built blood circulation system (27,28) and *in vivo* in the sagittal sinus in human brain. A 500 ms (longest saturation time available on the body coil) block shape RF pre-pulse with three different power levels ($2\ \mu T$, $3\ \mu T$ and $4\ \mu T$) was used, with frequency offsets from -75 ppm to 75 ppm with a step of 5 ppm. Signal intensities with (S_{sat}) and without (S_0) MT saturation pulse were acquired to determine the MT ratio ($\text{MTR} = 1 - S_{\text{sat}}/S_0$) for each combination of RF power levels and frequency offsets. Regions of interest (ROIs) were selected to cover almost the entire axial area in the blood phantom and the sagittal sinus territory in the brain. For convenience, we used bovine blood for the *ex vivo* measurement, as it is known that bovine blood has similar hemoglobin content, erythrocyte shape and size, and water permeability as human blood (29) and therefore is assumed to have similar longitudinal relaxation times and MT properties. Anticoagulation salt (20 mM sodium citrate) was added to fresh blood samples. Only blood with methemoglobin (metHb) level less than 2% was used. A normal hematocrit (Hct) level of 0.44 (30) was assured by adding either pure plasma or erythrocytes centrifuged (2500 rpm, 15 minutes) from the same blood sample. Blood was circulated at a velocity of approximately 3 ml/min to prevent erythrocyte precipitation. Blood oxygenation level was adjusted as described previously (31) and measured by a blood gas analyzer (Radiometer, Copenhagen, Denmark) before and after experiments. The averaged oxygenation level was reported. Only data with oxygenation level variation of less than 3% were analyzed. Blood pH was maintained at a normal physiological value of 7.4. Blood temperature was kept at 37°C using a warm water bath circulating around the blood tubes outside the coil and monitored by a fiber optic thermal sensor (FISO Technologies, Québec, Canada). All experiments were repeated for seven batches of bovine blood acquired on different days. Two blood oxygenation levels of $94\pm 3\%$ and $61\pm 2\%$ ($n = 7$), approximately corresponding to arterial and venous blood, were measured.

Human Studies

All subjects ($n = 7$) gave written informed consent before participating in this Johns Hopkins Institutional Review Board (IRB) approved, Health Insurance Portability and Accountability Act (HIPAA)-compliant study. To compare MT effects in brain tissue with those in blood, tissue MTR was measured *in vivo* as described above for the sagittal sinus. A T_1 map was acquired for each subject with an inversion recovery (IR) sequence using the same imaging parameters as above. Eight TIs were used (50, 100, 250, 500, 1000, 2000, 3000, 5000 ms). For each voxel, a three-parameter fit ($S = S(0)(1 - C \cdot e^{-TI/T_1})$) of the signal intensities as a function of TI was performed. Voxels with T_1 values of 700–850 ms and 1000–1300 ms (23) were used as masks for white matter (WM) and GM, respectively. Average MTR values for GM and WM were then calculated for each subject.

Functional MRI experiments were performed to detect neuronal activation in the primary visual cortex in human brain. All subjects ($n = 7$) in the MT effect experiments participated in the functional study. A black-white flashing checkerboard (frequency = 8 Hz, visual angle = 25°) was projected onto a screen in the back of the magnet for visual stimulation. An angled axial imaging slice was carefully selected to cover the primary visual cortex around the calcarine fissure. A block paradigm of 63 s stimulus-off was interleaved with 35 s stimulus-on. Each fMRI experiment session consisted of four stimuli blocks. Three functional VASO experiments were carried out for each subject using a spatially non-selective adiabatic inversion: 1) MT-VASO I with a CW MT pre-pulse of block shape, 500

ms length, $3\ \mu T$ power level irradiating at frequency offset 40 ppm; 2) MT-VASO II with a CW MT pre-pulse of block shape, 300 ms length, $3\ \mu T$ power level irradiating at frequency offset 40 ppm; 3) the original VASO experiment. The choice of the CW MT pre-pulses parameters was made based on the results discussed later. The scan time for each functional experiment was 7 minutes and 35 seconds during which 65 images were acquired. The specific absorption rates (SAR) shown on the scanner for experiments 1) and 2) were 0.9 W/kg and 0.8 W/kg respectively (which is well below the U.S. Food and Drug Administration (FDA) limit for the human brain).

fMRI Data Analysis

To correct for the potential subject motion during fMRI experiments, a standard 2D rigid body registration algorithm from Automated Image Registration (AIR) (32) was employed to co-register all images for each subject, after which a cubic spline interpolation algorithm was used to rectify the baseline drift of the time courses voxel by voxel. A ROI covering the primary visual cortex was carefully drawn for each subject. A statistical hypothesis test (two-tailed Z-test) with significance threshold of 0.01 was employed for activation detection within this ROI. Requirements for VASO voxel activation were $SNR > 20$ (26), Z-score < -2.5 and cluster size ≥ 4 . Note that the Z-score threshold is negative since VASO signal decreases during activation (6). The SNR threshold is necessary for reliable activation detection in VASO fMRI, as shown in a recent study (26). It has been reported that at least 10 s is needed for the CBV-based VASO hemodynamic response to return to baseline after stimulation (33). To keep the numbers of images approximately the same for on and off periods, the first 4 images in each off period and first image in each on period were not involved in activation detection.

Hemodynamic time courses were produced by averaging MR signals over all activated voxels and normalized to the average baseline signal. Only voxels activated in all three methods were used for further analysis. Ratios of average baseline (S) and equilibrium (S_0) signal intensities were determined. The noise level (N) was estimated by the standard deviation of a difference map from two baseline images in the middle of the second stimulus-off period. The SNR for each voxel was then defined as S/N and CNR was defined as $SNR \times |\Delta S/S| \times \sqrt{N_a}$ (N_a = number of images acquired during stimulus-on period that were involved in activation detection). Average SNR, Z-score, relative signal change ($\Delta S/S$) and CNR over all activated voxels were calculated. All programs were coded in Matlab 6.0 (Mathworks, Natick, MA, USA).

Results and Discussion

Simulations

In MT-VASO I, increasing the MT effect makes the signal after inversion closer to zero (less negative), thus leading to a higher tissue signal at TI. Greater MT effect can be achieved by increasing the power and/or duration of the RF pre-saturation pulse and/or decreasing the irradiating frequency offset with respect to the water resonance. Of course, the saturation power and duration are constrained by the MRI scanner hardware capacity and FDA guidelines. Saturation closer to water may cause more direct water saturation (21) that can not be ignored in blood, which will alter the blood nulling time. Considering these factors and based on the results in Table 1, we chose a block shape RF pulse of $3\ \mu T$ and 500 ms saturating at 40 ppm for MT-VASO I. In MT-VASO II the situation is more complicated. After inversion, when the tissue magnetization recovers under the influence of an MT saturation pulse, two effects compete (17–20). On one side the MT saturation pulse accelerates the inversion recovery process. On the other side, however, it also suppresses the tissue signal. Therefore the desirable MT effect that will result in the highest tissue signal at

blood nulling is not the greater the better. Keeping the same moderate power of $3 \mu T$ and frequency offset of 40 ppm as in MT-VASO I, we simulated the GM magnetization at TI of 1106 ms for saturation duration from 0–500 ms using a step size of 10 ms. Saturation times of 270–330 ms were found to furnish the maximum GM signal at this TI/TR combination. Therefore a $3 \mu T$, 300 ms RF pulse irradiating at 40 ppm was used in MT-VASO II. Fig. 1 shows the numerically simulated inversion recovery process for GM longitudinal magnetization in MT-VASO sequences I and II. At the blood nulling time, MT-VASO I and II produce about 37% and 35% extravascular tissue signal while the tissue signal in VASO is approximately 20%. The use of MT pre-pulses both before and after the inversion would furnish even greater tissue signal at TI. However, this would increase the SAR by more than 50%. When simulating a sequence with an MT saturation of $3 \mu T$ (at 40 ppm), and pulses of 500 ms before and 300 ms after inversion, the tissue signal (M_z/M_0) at the same nulling time was only about 1% higher than in MT-VASO I. This is not worthwhile with respect to the drastic increase in SAR.

MT Effects in Blood and Tissue

In Figure 2, the z-spectra of blood and tissue are compared. In Table 1, the MTR values of blood measured *ex vivo* in the blood phantom ($n = 7$) and *in vivo* in the sagittal sinus ($n = 7$) are compared with WM and GM MTRs. As expected, the MTR values reduce with frequency offset. There is 10–40% MT effect in tissue varying with saturation power and frequency offset. WM has more MT effect than GM ($P < 0.01$) at corresponding power and offset. For both *ex vivo* and *in vivo* blood, at frequency offsets of 40 ppm and 60 ppm, no significant difference is found between the MTR values of corresponding power levels ($P > 0.1$). In blood phantom studies, the MTR values were slightly smaller in blood of higher oxygenation level at corresponding power levels and frequency offsets (all $P < 0.5$). This can probably be attributed to the increase in blood T_2 relaxation time with elevating oxygenation level (31) which narrows the saturation line. T_1 does not change much with oxygenation (6,34). In human brain *in vivo*, the blood in the sagittal sinus is venous blood. So the MTR values are closer to *ex vivo* blood of oxygenation level $61 \pm 2\%$ at power of $2 \mu T$ and $3 \mu T$. At $4 \mu T$, the MTRs are larger possibly due to partial volume effects with tissue at the relatively low resolution used and the exchange between tissue and blood magnetization *in vivo*. The larger standard errors (SE) are probably the result of partial volume effects as well as of inter-subject variation. It can be seen from both *ex vivo* and *in vivo* results that when irradiating RF power is less than or equal to $3 \mu T$ and the frequency offset is larger than or equal to 40 ppm (pulse duration = 500 ms), the MT effect in blood is less than 2.5%. This small MT effect leads to approximately 20 ms difference in blood nulling time in MT-VASO I for TR from 2 s to 7 s, which is within accuracy range of the measured blood T_1 values (6,34). In MT-VASO II, where the saturation pulse duration is 200 ms shorter, even less MT effect is produced.

To verify experimentally that the two MT-VASO sequences can use the same blood nulling time as the original VASO method, the residual blood signal at the VASO nulling time was measured for each sequence both *ex vivo* in the blood phantom and *in vivo* in sagittal sinus in human brain (Table 2). The residual blood signal in the *in vivo* measurement was slightly higher ($P < 0.05$) than in the blood phantom, which we attribute to partial volume contamination. No significant difference was found between the three sequences. All residual blood signals are well below 0.5%, which is in the noise range, verifying that, under the current experimental settings, the same TI can be used for VASO and MT-VASO sequences.

Functional MRI experiments

Figs. 3a–c show representative activation maps for the MT-VASO and VASO sequences overlaid on the baseline images. Voxels in the sagittal sinus and other sulci have the typical dark VASO appearance, which verifies that the blood signal is close to zero. The ventricular signal was of comparable brightness in all sequences, which confirms that CSF has little MT effect (35). The data in Table 3 show that the MT-VASO sequences have higher signal intensity ($P < 0.01$) at the blood signal nulling time (S) with respect to the equilibrium signal (S_0). As a consequence, the SNR values for MT-VASO I and II are $47 \pm 8\%$ and $38 \pm 6\%$ higher, respectively, than in VASO, which is reflected by a higher number of activated voxels ($P < 0.01$), and improved average Z-score ($P < 0.01$). The mean hemodynamic time courses over all subjects ($n = 7$) are shown in Fig. 3d. It can be seen that the temporal responses as well as the relative signal changes ($\Delta S/S$) between rest and activation match for all three sequences. The signal change amplitude ($\sim 2\%$) was consistent with previous VASO studies of similar resolution and TR in human brains (6,26) and no significant difference was found in relative signal changes between the three sequences ($P > 0.1$). Since SNR increased while relative signal changes remain unchanged, the CNR was significantly enhanced ($P < 0.01$) by $44 \pm 12\%$ and $36 \pm 11\%$ for MT-VASO I and II, respectively, compared to the original VASO sequence. The data in Table 3 show that MT-VASO I is slightly more sensitive than MT-VASO II, giving higher tissue signal ($P < 0.01$), higher SNR ($P < 0.05$), more activated voxels ($P < 0.01$), and higher CNR ($P < 0.05$). MT-VASO I is also theoretically simpler with respect to designing optimal MT pre-pulse parameters in different experimental settings. MT-VASO II, on the other hand, is more time efficient because the MT saturation pulse is placed between inversion and MR acquisition.

It has been reported that tissue MTR decreases with neuronal depolarization (36,37). This MTR decrease has been attributed largely to a change in direct water saturation caused by the alteration in tissue T_2 during neuronal activation, as recently shown for pure water saturation by (38). The MT exchange process between bulk water and macromolecules was found to change negligibly during activation (36). In our MT-VASO method, when using the current offsets, the effect of direct water saturation changes on tissue MTR are expected to be negligible, which was experimentally confirmed in the absence of significant differences in relative signal change between VASO and MT-VASO sequences (Table 3).

More generally, MT-VASO can be performed with MT saturation at higher power levels ($> 3 \mu T$) and/or frequency offsets closer (< 40 ppm) to water resonance. In this case, the MT and direct saturation effect in blood can not be neglected and a different blood nulling time has to be calculated (MT-VASO I) or measured (MT-VASO II) for different TRs. This has the potential advantage that the MTR difference between blood and water becomes greater, which makes it possible to achieve more enhancement in tissue signal. On the other hand, however, the blood nulling time becomes shorter. Using the same MT model and the measured MT effects in this study, we simulated the residual tissue signal at TI with different saturation power and frequency offset for MT-VASO I. The greatest enhancement occurred around 20 ppm for each power. When TR was varied from 1 s to 7 s, we found that the shorter TR gave more relative enhancement. This would be very useful in view of VASO suffering low SNR especially at short TRs.

The energy deposition from the MT saturation pulses is a major limiting factor in all MT-related experiments. In this study a relatively long TR (7 s) was used mainly to suppress blood flow effects on VASO contrast (26). For such long TRs (5–7 s), the SAR reported on the scanner (< 1 W/kg) is well below the FDA limit. When using shorter TRs (2–3 s), SAR becomes larger (about 2 W/kg) but remains well within FDA limits. Power deposition can be reduced by employing pulsed MT (39) instead of CW saturation. When using TSE, the long 180° pulse train in the TSE readout also produces significant energy deposition. Using

less energy intensive readout approaches such as the gradient spin echo (GraSE) or basic EPI will mitigate the SAR problem at short TR and improve the temporal resolution for MT-VASO MRI or allow the use of stronger MT pulses.

The MT-VASO approach proposed in this paper can be combined with multi-slice VASO techniques, including the “multiple acquisitions with global inversion cycling” (MAGIC) approach (11,40). When using multi-slice MT experiments, two issues become important. The first is to remain within the SAR limit, which is well possible and automatically safeguarded by the manufacturer software. The second is for all slices to reach and maintain the same steady state when images are acquired. This can be done in a manner similar to the whole-brain pulsed MT technique proposed by Smith et al. (39).

Conclusions

It was shown that, when using moderate saturation power and a frequency offset sufficiently far from the water resonance (40 ppm or more), MT effects in tissue can be used to enhance the SNR and CNR of the VASO pulse sequence. This MT-VASO approach can be performed at the same blood nulling time as conventional VASO, because of negligible blood MT effects at these offsets. The SAR, which is always a concern for MT-related approaches, was well below the FDA limit for human brain for the moderate saturation power and time needed to accomplish the enhancement.

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Figure 1.

MT-VASO pulse sequences with an MT pre-pulse added before (MT-VASO I) and immediately after (MT-VASO II) the VASO inversion pulse along with the original VASO sequence are demonstrated. In all sequences, images are acquired at a TI when the blood signal is expected to be nulled. The recovery process of tissue (GM) magnetization was simulated using literature tissue parameters and the imaging parameters in the functional experiments.

Figure 2.

Z-spectra as a function of frequency offset of saturation with respect to water resonance (0 ppm) are shown. Error bars represent the standard errors over all batches of bovine blood ($n = 7$) in phantom and all subjects ($n = 7$) *in vivo*. Three saturation power levels were used: $2 \mu T$ (a,b), $3 \mu T$ (c,d) and $4 \mu T$ (e,f). It can be seen that blood has much less MT effect than GM tissue, especially at offsets greater than 20–40 ppm.

Figure 3.

Visual activation results in human brain at 3T. (a–c): Typical activation maps from one subject in MT-VASO sequences I, II and in VASO are shown in a–c, respectively. It can be seen that the tissue in MT-VASO I and II looks slightly brighter than in VASO, indicating higher signal for these equivalently scaled images. The hemodynamic responses from MT-VASO I (star, green), II (circle, blue) and VASO (square, red) are compared in (d). The two dotted vertical lines indicate the start and cessation of visual stimulus. The error bar is the standard error over all subjects ($n = 7$). The temporal profiles and response magnitudes of all three sequences match well within error.

Table 1

MTR* values (%) as a function of power and frequency offset in a bovine blood perfusion phantom and in sagittal sinus, GM, and WM in human brain *in vivo*.

	offset	20 ppm	40 ppm	60 ppm
	power [#]			
Blood phantom (Oxygenation 94±3%)	2μT	4.1±0.7	1.6±0.6	1.4±0.5
	3μT	5.4±0.6	2.1±0.9	1.7±0.6
	4μT	8.3±0.9	3.3±1.1	2.7±0.7
Blood phantom (Oxygenation 61±2%)	2μT	4.5±0.6	0.8±0.8	0.5±0.6
	3μT	6.0±0.6	2.3±0.6	1.9±0.7
	4μT	9.2±0.7	3.8±0.7	3.0±0.7
Sagittal sinus	2μT	-2.0±1.2	2.3±1.7	-0.9±4.0
	3μT	6.6±4.5	-0.3±9.0	1.0±6.2
	4μT	11.1±1.5	11.2±3.9	7.6±4.1
White matter	2μT	20.1±2.2	12.4±1.3	8.3±1.1
	3μT	31.7±2.7	20.2±2.1	15.3±1.6
	4μT	40.3±2.7	29.6±2.3	22.1±1.8
Gray matter	2μT	17.1±2.5	10.9±1.7	7.2±1.4
	3μT	27.8±3.2	18.7±2.7	13.3±2.0
	4μT	36.2±3.3	26.3±2.8	19.5±2.2

* Mean values ± standard error over all batches of bovine blood (n = 7) in phantom and all subjects (n = 7) *in vivo*.

[#] 500 ms RF pulse

Table 2

Residual blood signal* (%) at the VASO TI (1106ms for TR = 7s) used for blood nulling.

	MT-VASO I	MT-VASO II	VASO
Blood phantom (Oxygenation level 94±3%)	0.022±0.033	0.015±0.046	0.006±0.075
Blood phantom (Oxygenation level 61±2%)	0.046±0.048	0.119±0.036	0.046±0.067
Blood in sagittal sinus in human brain	0.104±0.140	0.133±0.111	0.120±0.132

* Mean values ± standard error over all batches of bovine blood (n = 7) in phantom and all subjects (n = 7) *in vivo*.

Residual tissue signal (S/S_0), voxel SNR, number of activated voxels, Z-score, relative signal change ($\Delta S/S$) during activation, and CNR in human brain at 3T for MT-VASO and VASO*.

Table 3

	S/S_0 (%)	SNR	Number of Activated Voxels	Z-score	$\Delta S/S$ (%)	CNR
MT-VASO I	37.8±5.6	89.5±4.2	931±56	-5.3±0.3	-1.9±0.4	7.1±0.7
MT-VASO II	33.1±4.8	83.2±3.1	737±67	-4.9±0.1	-1.8±0.6	6.6±0.6
VASO	21.3±3.2	60.2±7.7	503±98	-4.4±0.3	-2.0±0.5	4.8±0.6

* Mean values ± standard error over all subjects (n = 7).