Published in final edited form as:

Magn Reson Med. 2016 May ; 75(5): 1886–1893. doi:10.1002/mrm.25814.

¹⁷O Relaxation Times in the Rat Brain at 16.4T

Hannes M. Wiesner^{1,2}, Dávid Z. Balla¹, G. Shajan¹, Klaus Scheffler^{1,3}, Kâmil U urbil², Wei Chen², Kâmil Uluda ^{4,†}, and Rolf Pohmann^{1,†,*}

¹High-Field Magnetic Resonance Center, Max Planck Institute for Biological Cybernetics, 72076 Tübingen, Germany ²Center for Magnetic Resonance Research, Department of Radiology, University of Minnesota Medical School, Minneapolis, MN 55455, USA ³Department of Biomedical Magnetic Resonance, University of Tübingen, Tübingen, Germany ⁴Maastricht Brain Imaging Center (M-BIC), Department of Cognitive Neuroscience, Faculty of Psychology and Neuroscience, Maastricht University, Maastricht, The Netherlands

Abstract

Purpose—Measurement of the cerebral metabolic rate of oxygen (CMRO₂) via direct imaging of the ¹⁷O signal can be a valuable tool in neuroscientific research. However, knowledge of the longitudinal and transverse relaxation times of different brain tissue types is required, which is difficult to obtain because of the low sensitivity of natural abundance H₂¹⁷O measurements.

Methods—Using the improved sensitivity at a field strength of 16.4 T, relaxation time measurements in the rat brain were performed in vivo and postmortem with relatively high spatial resolutions, using a chemical shift imaging sequence.

Results—In vivo relaxation times of rat brain were found to be T₁ = 6.84 ± 0.67 ms and T₂* = 1.77 ± 0.04 ms. Postmortem H₂¹⁷O relaxometry at enriched concentrations after inhalation of ¹⁷O₂ showed similar T₂* values for gray (1.87 ± 0.04 ms) and white matter, significantly longer than muscle (1.27 ± 0.05 ms) and shorter than CSF (2.30 ± 0.16 ms).

Conclusion—Relaxation times of brain H₂¹⁷O were measured for the first time in vivo in different types of tissues with high spatial resolution. Since the relaxation times of H₂¹⁷O are expected to be independent of field strength, our results should help in optimizing the acquisition parameters for experiments also at other MRI field strengths.

Keywords

Relaxation times; brain water distribution; natural abundance oxygen-17; H₂¹⁷O; quadrupolar relaxation; X-Nuclei; quantification; spectroscopic imaging; MRSI; ultra-high field

*Corresponding Author: Dr. Rolf Pohmann, High-Field Magnetic Resonance Center, Max Planck Institute for Biological Cybernetics, Spemannstr. 41, 72076 Tübingen, Germany, Phone: +49 7071 601 903, Fax: +49 7071 601 702, rolf.pohmann@tuebingen.mpg.de.

†These authors contributed equally to this work.

Introduction

In vivo imaging of oxygen metabolism, a key biological parameter for characterizing the vital status of tissue, can have a large impact in medicine and neuroscience. While most established techniques used to measure the cerebral metabolic rate of oxygen (CMRO₂) have the limitations of being either indirect (e.g. calibrated BOLD (1,2)) or invasive (arterio-venous difference measurements (3–5) and PET (6)), MRI during catabolism of enriched ¹⁷O₂ to H₂¹⁷O can become a valuable alternative. However, the NMR detection of ¹⁷O is challenging due to its low gyromagnetic ratio (7), short transverse relaxation times (8) and low natural abundance (9). The sensitivity of ¹⁷O NMR has been shown to increase almost quadratically with field strength, supported by the field-independent relaxation times of unbound H₂¹⁷O (8,10,11). Further improvements can be realized by optimizing the acquisition parameters based on the relaxation times (12,13).

The relaxation mechanisms of the ¹⁷O nucleus are significantly different from those of protons due to the spin of +5/2 (7), generally being more intra-molecular as a result of quadrupolar interaction. Water as the exclusive source of the in vivo NMR signal of ¹⁷O as triplet (14–16) exhibits one peak in bulk water due to fast hydrogen exchange and isotropic molecular motion (17). At physiological conditions around neutral pH the relaxation of H₂¹⁷O is strongly influenced by chemical exchange and by temperature (15). Since field-dependent relaxation has been reported for bound water (18), a regional determination of representative relaxation times in complex in vivo tissue as a mixture of bound and free water in the brain is important for applying CMRO₂ imaging through H₂¹⁷O NMR detection.

In this study we determined the regional longitudinal T₁ and apparent transverse T₂* relaxation times of H₂¹⁷O at natural abundance and in enriched concentrations in the rat head at 16.4T. Due to the high magnetic field and optimized measurement parameters, local differences in the relaxation times among different tissue types of rats have been observed for the first time in vivo at relatively high spatial resolutions. Additional phantom measurements were performed for reference to assess the stability of the methods, owing to the influence of viscosity and chemical exchange by temperature (19).

Previous relaxometric studies of H₂¹⁷O in rat brain have not differentiated between different tissue types in vivo. Finally, since pH, temperature and concentrations of salts affect the chemical exchange and molecular motion, the linewidth of H₂¹⁷O is naturally auto-regulated in the brain within a narrow range. Therefore, the advantages of ultra-high field MRI make it possible to obtain the necessary signal sampling efficiency at sufficient spatial resolutions and the observed field independence of the H₂¹⁷O relaxation times ensures that these results can easily be applied to studies at other field strengths.

Methods

Instrumentation

Experiments were performed on a Bruker BioSpec Avance III system (Bruker Biospin MRI GmbH, Ettlingen, Germany) based on a 16.4 Tesla magnet (MagneX Scientific, Abingdon,

UK) with a 26 cm bore, equipped with gradients with an inner diameter of 12 cm and a maximum strength of 1 T/m within a rise time of 212 μ s (Resonance Research Inc., Billerica, MA, USA). Custom-built ^{17}O quadrature surface coils (diameter 1.5 cm; sensitive detection depth ~ 1 cm (20)) were tuned and matched for every sample individually to the ^{17}O Larmor-frequency of 94.6 MHz. The signals from 0° and 90° channels were combined by a quadrature hybrid and then amplified and digitized by a single-channel X-band receiver. Mutual coupling between the two channels, assessed by S21 measurements, was less than 20 dB. For proton imaging, a separate ^1H transmit and receive butterfly coil was attached on top of the ^{17}O coils.

Animal preparation

All procedures and experiments were approved by the local authorities and carried out by FELASA qualified personnel in compliance with the guidelines of the European Community (EUVD 86/609/EEC) for the care and use of laboratory animals. A total of 14 male Wistar rats (Charles River Laboratories, Sulzfeld, Germany) were used in this study. Animals were kept at a 12/12 hours day/night cycle with free access to nutrition and water.

In vivo measurements—Ten male Wistar rats were used for in vivo relaxometry measurements (T_1 : $n=5$; mean body weight 513 ± 71 g; T_2^* : $n=5$; mean body weight 529 ± 30 g). Anesthesia was induced in an induction chamber by 3-4% isoflurane (Forene®, Abbott, Chicago, IL, USA). During the preparation of the animal, anesthesia was maintained by spontaneous inhalation of room air with 2% isoflurane through an inhalation mask. A 22-gauge catheter (Venisystems Abbocath®, Abbott Laboratories, UK) was inserted into a lateral tail vein for intravenous infusion of medetomidine anesthesia. The rat head was fixated in a custom-built stereotaxic frame with the center of maximum sensitivity of the ^{17}O -coil in Z-direction close to the bregma of the rat skull. Lidocain spray (Xylocain®, AstraZeneca GmbH, Wedel, Germany) was applied topically before installation of the ear-bars. A subcutaneous bolus of Flunixin-Meglumin (5 mg/kg; Finadyne®, Essex Pharma GmbH, München, Germany) was given as analgesic for the intravenous catheter and fixation. The transition to continuous medetomidine anesthesia (Domitor®, Pfizer Inc., New York, NY, USA) was initiated by intravenous infusion at a rate of 0.1 mg/kg/h (dilution: 0.1 mg in 1 ml saline solution with 16 IU/ml of heparin [heparin-natrium 25000®, ratiopharm GmbH, Ulm, Germany]) with successive discontinuation of isoflurane over 15-20 minutes (21). For the maintenance of anesthetic efficacy during long experiments (22), the medetomidine infusion rate was increased every 75 minutes by 50 μ g/kg/h.

Breathing rate and exhaled respiration gases (Capstar-100 End-tidal CO_2 Analyzer, CWE Inc., Ardmore, PA, USA) were monitored and recorded (PowerLab & LabChart 5; ADInstruments, Australia). Rectally measured body temperature was maintained at $36.8 \pm 0.5^\circ\text{C}$ by an electric heating pad connected through a RF-filter. After the experiments, anesthesia was revoked using atipamezolhydrochlorid (Antisedan®, Pfizer Inc., New York, NY, USA).

Postmortem experiments—Four male Wistar rats (mean body weight 380 ± 131 g) were initially anesthetized with 3-4% and kept at 2% isoflurane during preparations. The animals

were orally intubated for mechanical ventilation and intravenously catheterized for infusion of alpha-chloralose at 50 mg/kg/h. Rats were positioned inside the magnet and were subjected to a total approx. 90 minutes inhalation of 30% O₂ with a ¹⁷O₂-enrichment of 70% (Nukem GmbH, Germany) in N₂O (one rat in N₂) as part of a different study. Two hours after inhalation, the animals were euthanized with >5% isoflurane without changing their position within the magnet. Acquisitions started 10 minutes after exitus with the rat body allowed to equilibrate to ambient temperature in the magnet bore (21 °C at isocenter) (23).

MRI experiments

Adjustments

The RF-power for a nominal 90° excitation at the ¹⁷O frequency was calibrated globally for each individual sample by maximizing the signal of a “pulse-and-acquire” sequence, using a 50 μs on-resonance block-pulse. For anatomical imaging and shimming, a proton surface coil was mounted on top of the ¹⁷O coil assembly, while in some of the in vivo measurements a ¹H resonator surrounding the setup was used instead. First and second order B₀ shims were adjusted using FASTMAP (24) with a 1 ms Gaussian pulse. Proton images were acquired at high in-plane resolutions with equal slice thickness as the corresponding H₂¹⁷O images.

For in vivo H₂¹⁷O imaging, a 3D chemical shift imaging (CSI) sequence using weighted averaging of the k-space was applied (25,26). To obtain sufficient signal in spite of the short relaxation time, the acquisition delay between the middle of the excitation pulse and the start of the acquisition was minimized to 484 μs. Since a non-selective excitation pulse was used, the sensitive region in the field of view (FOV) of the 3D CSI sequence with FID detection was determined by the profile of the surface coil. The raw data was Fourier transformed along the three spatial dimensions, resulting in localized FIDs, which were used to estimate T₂* times. Subsequent Fourier transformation of the time domain resulted in localized spectra. The amplitudes of the phased water peaks were used to construct the final H₂¹⁷O images.

Phantom measurements

Reference relaxation time measurements on pure water with 20 ml of a 10%-enriched H₂¹⁷O water phantom (Nukem GmbH, Germany) in a glass tube were performed at room temperature.

T₁ and T₂* were measured as described below. A ROI in the center of the phantom was selected in order to avoid potential effects of superficial heating owing to the specific absorption rate (SAR) of RF-power and to attenuate susceptibility effects in peripheral regions of the phantom.

T₁ relaxometry

Inversion recovery images were acquired with 20 inversion delays ranging from 0.5 ms to 45 ms in logarithmically increasing steps, using an adiabatic hyperbolic secant pulse (duration 0.5 ms, bandwidth 10 kHz) for inversion. Imaging parameters were: FOV 30 × 15.71 × 27

mm³; matrix size 21 × 11 × 9 (nominal voxel volume 6.12 μl); relaxation delay 45 ms (TR ≫ 5 T₁); 50 μs pulse for excitation with a nominal flip angle of 90°. 10240 FIDs for each inversion step were acquired within 7-15 minutes (depending on the inversion time) with a maximum of 41 averages in the center of k-space. Spectroscopic data of 1250 points per FID was recorded within 10 ms with a bandwidth of 125 kHz (1321 ppm).

T₁ was determined by a mono-exponential fit of the intensity of selected voxels, using standard non-linear least-squares algorithms (Curve Fitting Toolbox, MATLAB®, The Mathworks Inc., Natick, MA, USA). The signal intensities for short inversion times were inverted while measurements within one standard deviation around the noise level were excluded before fitting.

The different tissue types were classified based on the proton images with reference to a rat brain atlas (27). At least two voxels between different tissue regions were not included along the outer boundary of ROIs to avoid partial volume contaminations due to the large voxel sizes in the H₂¹⁷O measurements. The T₁-measurement covered a region of 0.9 to 1.5 cm in Z-direction (depending on the size of the rat), and thus most of each rat brain. Only voxels inside the brain with a sufficient spectral SNR>12 were fitted, which resulted in a ratio of 56% brain vs. 44% muscle voxels on average.

T₂* relaxometry in vivo

For T₂* quantification, a short TR of 4.9 ms and a flip angle of 67.5°, close to the expected Ernst angle for the rat brain (FA=64° for T₁ at 17.6 T (28)) was used to sample 3D CSI with a FOV of 27.5 × 12.5 × 22 mm³ and a 39 × 17 × 15 matrix (nominal voxel volume 0.76 μl). Three million FIDs (375 spectral points, bandwidth 100 kHz, 2811 averages in the center of k-space) were acquired within 4 hours and 6 minutes from each rat.

The real part of the Fourier-transformed FID was frequency-corrected by adjusting the phase in the time-domain and fitted against a mono-exponential decay. Voxel-wise estimated relaxation times were averaged over manually selected ROIs for each tissue type. Extrapolating the FIDs to zero excitation and acquisition delay resulted in T₁-weighted images, after normalizing the intensity by correcting for B₁ inhomogeneity as determined in a phantom (29).

T₂* relaxometry postmortem

For high-resolution anatomical images and T₂* quantification, a CSI-sequence without k-space weighting with a TR of 12 ms and a flip angle of 90° was used. To avoid RF-induced heating, the excitation pulse length was increased to 400 μs to compensate for the lack of the cooling effect of blood circulation.

A FOV of 27.5 × 12.5 × 25 mm³ was sampled with a matrix of 41 × 19 × 25 voxels (voxel volume 0.44 μl). Approximately 2.5 million FIDs with 1000 points each and a spectral bandwidth of 100 kHz were acquired within 8 hours and 18 minutes. Post-processing for T₂* was then performed as in vivo.

Relaxation times are given as mean value $\pm$ standard deviation over all voxels of the selected region, with the nominal volume of the ROI added in brackets, if applicable.

Results

Phantom measurements

Phantom measurements on the 10%-enriched H_2^{17}O water sample resulted in a T_1 value of 6.24 ± 0.37 ms over the sensitive region of the surface coil, indicating stable T_1 -quantification and an efficient inversion over the entire FOV. In the same phantom, a T_2^* of 2.24 ± 0.09 ms was found.

In vivo relaxation times T_1

T_1 -values for all rats are shown in Table 1. The T_1 of brain tissue (gray and white matter) was 6.84 ± 0.67 ms and significantly higher than that of muscle (5.67 ± 1.12 ms). Muscle tissue could be clearly differentiated from brain in image intensity (Fig. 1b) and relaxation times (Fig. 1c). No significant intra-cerebral contrast was observed at this resolution. A repeated measurement in the same rat in a different session demonstrated highly reproducible T_1 -values (Table 1; rat 5).

In vivo and postmortem relaxation times T_2^*

In the high-resolution H_2^{17}O images, intra-cortical structures were consistently visible due to the decreased image intensity of white matter (i.e. corpus callosum and optic radiation) against cortical gray matter in all animals, both in vivo and postmortem. T_2^* of brain tissue had a mean value of 1.77 ± 0.04 ms and did not vary significantly between white and gray matter (Fig. 2c, Table 2a), while being significantly increased in CSF, e.g. in the ventricles (in vivo: 2.21 ± 0.20 ms; postmortem: 2.30 ± 0.16 ms, Table 2). A T_2^* of ~ 4 ms was measured in the eyes, too small in volume for quantitative evaluation. No significant deviation from a single exponential relaxation behavior was apparent in the signal time course of the localized FIDs as used for the T_2^* estimations (Fig. 3a). Extrapolation to $\text{TE}=0$ yielded combined spin density- and T_1 -weighted images (Fig. 3c), showing highest signal intensity in muscle tissue and lowest in CSF and ventricles, confirming the lower T_1 in muscle than in brain (Fig. 1c).

Rats undergoing $^{17}\text{O}_2$ -inhalations which were used for the postmortem T_2^* measurements showed a global increase of the image intensity by a factor of 4 to 5 compared to the natural abundance H_2^{17}O signal, due to significant metabolic conversion of $^{17}\text{O}_2$ to H_2^{17}O . No intra-cortical difference from metabolic rates (e.g. between white and gray matter) was detectable, due to the long duration of anatomical acquisition (i.e. diffusion of locally elevated H_2^{17}O concentration in equilibrium due to exhaustion of concentration gradients). Postmortem T_2^* values in muscle, GM and WM tissue were slightly increased compared to in vivo data, whereas the difference between brain tissue and CSF remained similar (Table 2b). This was accompanied by a generally decreased image contrast in the postmortem measurements, especially in regions with high water content (Fig. 4).

Discussion

In this study, we have taken advantage of the increased signal at ultra-high magnetic field to quantify H_2^{17}O relaxation times in the rat brain in vivo, for the first time differentiating different tissue types with relatively high spatial resolution. The results of this study should be beneficial to design future experiments, but also to assess the results of previous quantitative examinations. While a spectroscopic technique was used in all acquisitions to reach high sensitivity for the rats with and without $^{17}\text{O}_2$ inhalation, no metabolite peaks except the water signal were observable in our spectra (30–34) because of the limited excitation bandwidth in relation to the large range of chemical shifts with molecules containing ^{17}O .

Phantom studies

Our phantom measurements are in relative agreement with results from previous non-localized measurements which reported values of T_1 around 6–8 ms for enriched pure water (35) at physiological temperatures. Temperature controlled T_2^* measurements using continuous wave NMR reported T_2^* up to 4 ms at enriched H_2^{17}O concentrations (36,37) in contrast to our significantly lower values, which could be a result of the higher achievable B_0 field-homogeneity in non-localized NMR spectroscopy, of better temperature control in the non-localized measurements in NMR spectrometers and higher purity from paramagnetic ions of the glassware being used (35). Furthermore, a fair amount of one-dimensional acquisitions in the 1970s accumulated in vitro estimations of muscle tissue T_1 and T_2 (37–39). In contrast to proton MRI, T_2^* contrast changes close to air-sample borders were not observed in any images nor with varying voxel sizes as the frequency of H_2^{17}O at 16.4 Tesla used in this study translates to a Larmor frequency of 2.2 Tesla for protons. In addition, peak intensity changed by less than 15% comparing unlocalized spectra with and without B_0 -shimming, (i.e. with a smaller change in T_2^*).

Tissue specific relaxation and anatomical contrast

The differences between muscle and brain tissue H_2^{17}O relaxation times have similar proportions as those known from proton measurements (40–42) and cannot be explained from temperature difference alone. However, intra-cortical differences are much less pronounced than for proton MRI due to the limited influence of the inter-molecular environment on the field-independent relaxation of H_2^{17}O (43). The lack of differentiable T_2^* relaxation times between gray and white matter and the clear separation by low intensity of white matter structures indicate a difference in either T_1 or difference in H_2^{17}O concentration (Fig. 3c). The latter could be due to the variations in water content of more than 15% between gray and white matter as found in the human brain (44) and could affect natural abundance H_2^{17}O calibration (45).

The higher intersubject variability of the T_1 measurements compared to the T_2^* data (Table 1 vs. 2) is mainly due the lower sensitivity of the inversion recovery sequence compared to the simple FID acquisition used to determine T_2^* . In part, choosing larger voxels for the T_1 measurements compensated this. However, the low sensitivity of H_2^{17}O in general requires generally multiple repetitions for averaging the signal both in T_1 and T_2^* measurements,

which is possible due to the generally short T_1 in contrast to longer relaxing nuclei like protons.

Most previous investigations on oxygen consumption were often limited in localization because of the low sensitivity of the ^{17}O nucleus, thus neglecting potential contributions from non-brain tissue. In animal studies using surface coils for detection, the signal from muscles could cause significant confound, owing to the property of small coils to overemphasize superficial (i.e. proximal) anatomical structures. However, our results indicate that the low T_2^* values in muscle tissues presented here assure that the signal contribution from this tissue type will play a less dominating role when sufficiently long TE is used.

While the still relatively large voxels sizes may lead to residual partial volume effects, further increasing the spatial resolution, which is constricted by the low SNR of the measurements, will be challenging. In our experiments we have chosen a CSI sequence because of its high SNR efficiency and its short acquisition delay. The necessary SNR gain for improving the resolution might be possible by further reducing the signal loss due to transverse relaxation by using imaging sequences with ultrashort or even zero TE.

Comparison of in vivo and postmortem

Differences between in vivo and postmortem relaxation times reflect well known effects from previous studies (e.g. due to cell rupture, increased diffusion between intra- and extracellular space and differences in temperature (23,46)). Temperature changes due to the rapid succession of RF-pulses can distort the resulting relaxation times especially in the post-mortem measurements, where the body temperature is lower and no perfusion dissipates the heat. While SAR was reduced in these measurements by increasing the pulse duration, regions close to the coil, especially in the skeletal muscles, may heat up, explaining the slightly higher T_2^* in those regions. In the ex vivo measurements, it was possible to reach even higher spatial resolutions due to the H_2^{17}O enrichment and the long measurement times.

^{17}O relaxation times across field strength

In contrast to proton MRI, the longitudinal and transverse relaxation times of H_2^{17}O are expected to be independent of field strength. Figure 5 shows an overview of previously published T_2^* -values at different field strengths (8,47–50), including the results from the current study. All T_2^* values are remarkably close around 1.8 ms for brain tissue, because it is maintained in an auto-regulatory regime of temperature and pH, some of the most dominant factors for transverse H_2^{17}O relaxation. This data is consistent with the assumption of field-independent relaxation times, albeit a slight tendency to shorter T_2^* (Fig. 5) might be visible in muscle tissue.

Differences to previous studies might be due to differences in temperature, which may result from convection effects or the different temperature controlling procedures. They can also be caused by RF-heating in in vivo experiments, since the short T_1 and T_2^* and the necessity of rapid averaging increase SAR. For ^{17}O experiments at high fields, SAR monitoring and reduction is critical especially with humans. Thus, in the postmortem study we used longer

RF excitation pulses to avoid excessive heating at the expense of signal loss due to relaxation during the pulse. Further discrepancies between the current study and previous experiments may be due to differences in the sampled tissue, since most previous in vivo data were acquired with limited spatial localization and might represent a different mixture of tissue types.

Conclusion

The use of ultra-high field strength made it possible to determine tissue specific ^{17}O relaxation parameters. These might be used for sequence optimization (13,51) and analysis of data acquired using short TR during inhalation of enriched $^{17}\text{O}_2$ gas for the determination of CMRO $_2$. Due to the apparent field independence of the ^{17}O relaxation times, the results can easily be transferred to lower field strengths, as long as tissue and temperature differences are taken into account.

Acknowledgments

The study was funded by the Max Planck Society and supported in part by NIH grants: NS41262, NS070839 and P41 RR08079. We thank Hellmut Merkle and Michael Beyerlein for advice on RF-coils and animal setup.

References

1. Davis TL, Kwong KK, Weisskoff RM, Rosen BR. Calibrated functional MRI: Mapping the dynamics of oxidative metabolism. *Proc Natl Acad Sci USA*. 1998; 95:1834–1839.10.2307/44371 [PubMed: 9465103]
2. Hoge RD, Atkinson J, Gill B, Crelier GR, Marrett S, Pike GB. Linear coupling between cerebral blood flow and oxygen consumption in activated human cortex. *Proc Natl Acad Sci USA*. 1999; 96:9403–9408.10.1073/pnas.96.16.9403 [PubMed: 10430955]
3. Gotoh F, Meyer JS, Takagi Y. Cerebral effects of hyperventilation in man. *Arch Neurol*. 1965; 12:410–423.10.1001/archneur.1965.00460280080008 [PubMed: 14264873]
4. Gotoh F, Meyer JS, Ebihara S. Continuous recording of human cerebral blood flow and metabolism: methods for electronic monitoring of arterial and venous gases and electrolytes. *Med Res Eng*. 1966; 5:13–19. [PubMed: 5937073]
5. Meyer JS, Gotoh F, Favale E. Cerebral metabolism during epileptic seizures in man. *Electroencephalogr Clin Neurophysiol*. 1966; 21:10–22.10.1016/0013-4694(66)90054-X [PubMed: 4165360]
6. Mintun MA, Raichle ME, Martin WRW, Herscovitch P. Brain Oxygen Utilization Measured with O-15 Radiotracers and Positron Emission Tomography. *J Nucl Med*. 1984; 25:177–187. [PubMed: 6610032]
7. Alder F, Yu FC. On the Spin and Magnetic Moment of ^{17}O . *Phys Rev*. 1951; 81:1067–1068.10.1103/PhysRev.81.1067
8. Zhu X-H, Merkle H, Kwag J-H, Ugurbil K, Chen W. ^{17}O relaxation time and NMR sensitivity of cerebral water and their field dependence. *Magn Reson Med*. 2001; 45:543–549.10.1002/mrm.1073 [PubMed: 11283979]
9. Berglund M, Wieser ME. Isotopic compositions of the elements 2009 (IUPAC Technical Report). *Pure Appl Chem*. 2011; 83:397–410.10.1351/PAC-REP-10-06-02
10. Thelwall, PE.; Blackband, SJ.; Chen, W. Field dependence of ^{17}O T1, T2 and SNR - in vitro and in vivo studies at 4.7, 11 and 17.6 Tesla. *Proceedings of the 11th Annual Meeting of ISMRM*; Toronto, ON, Canada. 2003. p. 504
11. Lu M, Zhang Y, Ugurbil K, Chen W, Zhu X-H. In vitro and in vivo studies of ^{17}O NMR sensitivity at 9.4 and 16.4 T. *Magn Reson Med*. 2013; 69:1523–1527.10.1002/mrm.24386 [PubMed: 22777729]

12. Becker ED, Ferretti JA, Gambhir PN. Selection of optimum parameters for pulse Fourier transform nuclear magnetic resonance. *Anal Chem.* 1979; 51:1413–1420.10.1021/ac50045a016
13. Pohmann R, von Kienlin M, Haase A. Theoretical Evaluation and Comparison of Fast Chemical Shift Imaging Methods. *J Magn Reson.* 1997; 129:145–160.10.1006/jmre.1997.1245 [PubMed: 9441879]
14. Meiboom S. Nuclear Magnetic Resonance Study of the Proton Transfer in Water. *J Chem Phys.* 1961; 34:375–388.10.1063/1.1700960
15. Hertz HG, Klute R. The Slowing Down of Proton Exchange in Aqueous Solutions of Structure Breaking Ions. *Z Phys Chem (N F).* 1970; 69:101–107.10.1524/zpch.1970.69.1_2.101
16. Okroyan G, Kushnarev D, Kalabin G, Proidakov A. Spin–Spin Relaxation in the 17O NMR Spectra of Na⁺ and K⁺ Water Clusters. *J Struct Chem.* 2002; 43:242–245.10.1023/A:1019640205710
17. Redfield AG. On the Theory of Relaxation Processes. *IBM J Res Dev.* 1957; 1:19–31.10.1147/rd.11.0019
18. Denisov VP, Halle B. Thermal Denaturation of Ribonuclease A Characterized by Water 17O and 2H Magnetic Relaxation Dispersion. *Biochemistry.* 1998; 37:9595–9604.10.1021/bi980442b [PubMed: 9649343]
19. Boykin, DW. 17O NMR spectroscopy in organic chemistry. CRC press; 1991.
20. Kumar A, Bottomley P. Optimized quadrature surface coil designs. *MAGMA.* 2008; 21:41–52.10.1007/s10334-007-0090-2 [PubMed: 18057975]
21. Weber R, Ramos-Cabrer P, Wiedermann D, van Camp N, Hoehn M. A fully noninvasive and robust experimental protocol for longitudinal fMRI studies in the rat. *NeuroImage.* 2006; 29:1303–1310.10.1016/j.neuroimage.2005.08.028 [PubMed: 16223588]
22. Pawela CP, Biswal BB, Hudetz AG, Schulte ML, Li R, Jones SR, Cho YR, Matloub HS, Hyde JS. A protocol for use of medetomidine anesthesia in rats for extended studies using task-induced BOLD contrast and resting-state functional connectivity. *NeuroImage.* 2009; 46:1137–1147.10.1016/j.neuroimage.2009.03.004 [PubMed: 19285560]
23. Fagan AJ, Mullin JM, Gallagher L, Hadley DM, Macrae IM, Condon B. Serial postmortem relaxometry in the normal rat brain and following stroke. *J Magn Reson Imaging.* 2008; 27:469–475.10.1002/jmri.21246 [PubMed: 18219613]
24. Gruetter R. Automatic, localized in Vivo adjustment of all first-and second-order shim coils. *Magn Reson Med.* 1993; 29:804–811.10.1002/mrm.1910290613 [PubMed: 8350724]
25. Brown TR, Kincaid BM, Ugurbil K. NMR chemical shift imaging in three dimensions. *Proc Natl Acad Sci USA.* 1982; 79:3523–3526.10.2307/12315 [PubMed: 6954498]
26. Pohmann R, von Kienlin M. Accurate phosphorus metabolite images of the human heart by 3D acquisition-weighted CSI. *Magn Reson Med.* 2001; 45:817–826.10.1002/mrm.1110 [PubMed: 11323808]
27. Paxinos, G.; Watson, C. *The Rat Brain in Stereotaxic Coordinates: Hard Cover Edition.* Academic Press; 2006.
28. Thelwall PE. Detection of 17O-tagged phosphate by 31P MRS: A method with potential for in vivo studies of phosphorus metabolism. *Magn Reson Med.* 2007; 57:1168–1172.10.1002/mrm.21226 [PubMed: 17534916]
29. Axel L, Costantini J, Listerud J. Intensity correction in surface-coil MR imaging. *AJR Am J Roentgenol.* 1987; 148:418–420.10.2214/ajr.148.2.418 [PubMed: 3492123]
30. Irving CS, Lapidot A. Haemoglobin-17O2 Revisited. *Nature.* 1971; 230:224–224.10.1038/10.1038/newbio230224a0 [PubMed: 4926711]
31. Oldfield E, Lee HC, Coretsopoulos C, Adebodun F, Park KD, Yang S, Chung J, Phillips B. Solid-state oxygen-17 nuclear magnetic resonance spectroscopic studies of 17O2 picket fence porphyrin, myoglobin, and hemoglobin. *J Am Chem Soc.* 1991; 113:8680–8685.10.1021/ja00023a015
32. Lee HC, Oldfield E. Oxygen-17 nuclear magnetic resonance-spectroscopic studies of carbonmonoxy hemoproteins. *J Am Chem Soc.* 1989; 111:1584–1590.10.1021/ja00187a009
33. Gerothanassis IP. 17O NMR studies of hemoproteins and synthetic model compounds in the solution and solid states. *Prog Nucl Magn Reson Spectrosc.* 1994; 26(Part 3):239–292.10.1016/0079-6565(94)80008-1

34. De Graaf RA, Brown PB, Rothman DL, Behar KL. Natural abundance ^{17}O NMR spectroscopy of rat brain in vivo. *J of Magn Reson*. 2008; 193:63–67.10.1016/j.jmr.2008.04.019 [PubMed: 18456525]
35. Hindman JC, Svirnickas A, Wood M. Spin-lattice relaxation of oxygen-17 in water. *J Phys Chem*. 1970; 74:1266–1269.10.1021/j100701a018
36. Garrett BB, Denison AB, Rabideau SW. Oxygen-17 relaxation in water. *J Phys Chem*. 1967; 71:2606–2611.10.1021/j100867a033
37. Swift TJ, Barr EM. An Oxygen Magnetic Resonance Study of Water in Frog Skeletal Muscle. *Ann N Y Acad Sci*. 1973; 204:191–196.10.1111/j.1749-6632.1973.tb30779.x
38. Civan MM, Shporer M. ^{17}O Nuclear Magnetic Resonance Spectrum of H_2^{17}O in Frog Striated Muscle. *Biophys J*. 1972; 12:404–413.10.1016/S0006-3495(72)86092-2 [PubMed: 4537032]
39. Fung BM, McGaughy TW. Study of spin-lattice and spin-spin relaxation times of ^1H , ^2H , and ^{17}O in muscular water. *Biophys J*. 1979; 28:293–303.10.1016/S0006-3495(79)85177-2 [PubMed: 233613]
40. Crmillieux Y, Ding S, Dunn JF. High-resolution in vivo measurements of transverse relaxation times in rats at 7 Tesla. *Magn Reson Med*. 1998; 39:285–290.10.1002/mrm.1910390216 [PubMed: 9469712]
41. Stanis GJ, Odrobina EE, Pun J, Escaravage M, Graham SJ, Bronskill MJ, Henkelman RM. T_1 , T_2 relaxation and magnetization transfer in tissue at 3T. *Magn Reson Med*. 2005; 54:507–512.10.1002/mrm.20605 [PubMed: 16086319]
42. Pohmann R, Shajan G, Balla DZ. Contrast at high field: Relaxation times, magnetization transfer and phase in the rat brain at 16.4 T. *Magn Reson Med*. 2011; 66:1572–1581.10.1002/mrm.22949 [PubMed: 21671265]
43. Glasel JA. A Study of Water in Biological Systems by ^{17}O Magnetic Resonance Spectroscopy, II. Relaxation Phenomena in Pure Water. *Proc Natl Acad Sci USA*. 1967; 58:27–33.10.2307/58166 [PubMed: 5231608]
44. Whittall KP, Mackay AL, Graeb DA, Nugent RA, Li DKB, Paty DW. In vivo measurement of T_2 distributions and water contents in normal human brain. *Magn Reson Med*. 1997; 37:34–43.10.1002/mrm.1910370107 [PubMed: 8978630]
45. Atkinson IC, Thulborn KR. Feasibility of mapping the tissue mass corrected bioscale of cerebral metabolic rate of oxygen consumption using ^{17}O -oxygen and ^{23}Na MR imaging in a human brain at 9.4 T. *NeuroImage*. 2010; 51:723–733.10.1016/j.neuroimage.2010.02.056 [PubMed: 20188194]
46. Ith M, Bigler P, Scheurer E, Kreis R, Hofmann L, Dirnhofer R, Boesch C. Observation and identification of metabolites emerging during postmortem decomposition of brain tissue by means of in situ ^1H -magnetic resonance spectroscopy. *Magn Reson Med*. 2002; 48:915–920.10.1002/mrm.10294 [PubMed: 12418008]
47. Fiat D, Ligeti L, Lyon RC, Ruttner Z, Pekar J, Moonen CTW, McLaughlin AC. In vivo ^{17}O NMR study of rat brain during $^{17}\text{O}_2$ inhalation. *Magn Reson Med*. 1992; 24:370–374.10.1002/mrm.1910240218 [PubMed: 1569875]
48. Fiat D, Dolinsek J, Hankiewicz J, Dujovny M, Ausman J. Determination of regional cerebral oxygen consumption in the human: ^{17}O natural abundance cerebral magnetic resonance imaging and spectroscopy in a whole body system. *Neurol Res*. 1993; 15:237–248. [PubMed: 8105403]
49. Pekar J, Sinnwell T, Ligeti L, Chesnick AS, Frank JA, McLaughlin AC. Simultaneous Measurement of Cerebral Oxygen Consumption and Blood Flow Using ^{17}O and ^{19}F Magnetic Resonance Imaging. *J Cereb Blood Flow Metab*. 1995; 15:312–320.10.1038/jcbfm.1995.36 [PubMed: 7860664]
50. Borowiak R, Groebner J, Haas M, Hennig J, Bock M. Direct cerebral and cardiac ^{17}O -MRI at 3 Tesla: initial results at natural abundance. *MAGMA*. 2014; 27:95–99.10.1007/s10334-013-0409-0 [PubMed: 24077796]
51. Ernst RR, Anderson WA. Application of Fourier Transform Spectroscopy to Magnetic Resonance. *Rev Sci Instrum*. 1966; 37:93–102.10.1063/1.1719961

Figure 1. Longitudinal relaxation time T_1

a) Proton FLASH image of a coronal slice in the rat head in vivo.

b) Signal intensity of the H_2^{17}O spectral peak at natural abundance concentration of the same slice with delineations of forebrain (yellow) and surrounding muscle (red).

c) Corresponding slice of H_2^{17}O in vivo T_1 relaxation times with clear differences between brain and muscle tissue. A 5 mm scale bar (green) is shown for the colocalized slices.

Figure 2. Apparent in vivo transverse relaxation time T_2^*

- a) Coronal FLASH proton image.
- b) CSI anatomical contrast of rat tissues in image intensity of $H_2^{17}O$ with intra-cortical differentiations of white matter and ventricles.
- c) T_2^* estimates of $H_2^{17}O$ with pronounced differentiation inside brain parenchyma (gray matter, white matter) and cerebrospinal fluid in the ventricles.

Figure 3.

- a) Semi-logarithmic plot of the MR-signal (real part) averaged over ROIs of one rat from the in vivo measurements with fitted T_2^* relaxation curves for four tissue types. Free-induction-decays of different tissue types show high linearity without pronounced modulations.
- b) Reconstructed image of muscle and brain tissue based on the spectral peak of $H_2^{17}O$.
- c) T_1 -weighted image generated by extrapolation of the FIDs (see text) to an acquisition delay of $t=0$ for the same coronal slice as shown in b).

Figure 4. Apparent postmortem transverse relaxation time T_2^*

a) Postmortem images with increased $H_2^{17}O$ concentration due to metabolic enrichment after inhalation of $^{17}O_2$ gas. Olfactory bulb, forebrain, thalamus and cortex are shown with clear contrast between white matter (corpus callosum) and ventricles.

b) T_2^* map of the same slices as in a) exhibiting increased relaxation times especially in cortical fluids of the ventricles.

Figure 5. Literature comparison of T₂* relaxation times at different B₀ field strength of in vivo brain tissue. Span of error bar equals standard deviation, if available.

Table 1

In vivo T_1 relaxation times of natural abundance $H_2^{17}O$ measured in the rat head.

In vivo T_1	Muscle tissue	Brain tissue
rat 1 (583 g)	5.25 ± 1.02 (324 μ l) *	6.34 ± 0.67 (477 μ l) *
rat 2 (415 g)	6.47 ± 1.15 (459 μ l)	6.84 ± 0.62 (441 μ l)
rat 3 (483 g)	5.86 ± 1.10 (275 μ l) *	7.64 ± 0.52 (282 μ l) *
rat 4 (580 g)	5.22 ± 0.84 (214 μ l) *	6.58 ± 0.39 (398 μ l) *
rat 5 (504 g) #1	5.45 ± 0.71 (312 μ l) *	6.79 ± 0.40 (398 μ l) *
rat 5 (504 g) #2	5.74 ± 0.70 (312 μ l) *	6.83 ± 0.45 (398 μ l) *
Pop. Mean	5.67 ± 1.12 *	6.84 ± 0.67 *

* $p < 0.05$ significant population difference between muscle and brain tissue (paired t-test).

Table 2

In vivo and postmortem T_2^* relaxation times of $H_2^{17}O$ measured in the rat head.

a) In vivo T_2^* (natural abundance)				
	Muscle tissue	WM	GM	CSF
rat A (567 g)	1.03 ± 0.18 (309 µl)	1.86 ± 0.15 (24 µl)	1.83 ± 0.13 (111 µl)	2.56 ± 0.23 (38 µl)
rat B (499 g)	1.07 ± 0.18 (296 µl)	1.78 ± 0.09 (29 µl)	1.75 ± 0.08 (126 µl)	2.19 ± 0.12 (27 µl)
rat C (503 g)	1.08 ± 0.17 (301 µl)	1.75 ± 0.11 (25 µl)	1.73 ± 0.10 (128 µl)	2.13 ± 0.16 (28 µl)
rat D (526 g)	1.15 ± 0.16 (361 µl)	1.71 ± 0.10 (20 µl)	1.77 ± 0.08 (148 µl)	2.14 ± 0.11 (27 µl)
rat E (552 g)	1.27 ± 0.15 (315 µl)	1.77 ± 0.08 (14 µl)	1.76 ± 0.15 (100 µl)	2.05 ± 0.11 (10 µl)
Pop. Mean [ms]	1.12 ± 0.09[*]	1.77 ± 0.06	1.77 ± 0.04	2.21 ± 0.20[*]
b) Postmortem T_2^* (enriched ~4-5 times of natural abundance)				
rat F (250 g)	1.30 ± 0.21 (232 µl)	1.81 ± 0.17 (33 µl)	1.83 ± 0.15 (199 µl)	2.27 ± 0.35 (14 µl)
rat G (430 g)	1.28 ± 0.16 (239 µl)	1.84 ± 0.21 (25 µl)	1.85 ± 0.17 (182 µl)	2.26 ± 0.39 (9 µl)
rat H (540 g)	1.29 ± 0.25 (276 µl)	1.91 ± 0.33 (24 µl)	1.92 ± 0.21 (102 µl)	2.14 ± 0.44 (8 µl)
rat I (300 g)	1.20 ± 0.16 (207 µl)	1.87 ± 0.16 (22 µl)	1.89 ± 0.12 (175 µl)	2.53 ± 0.35 (25 µl)
Pop. Mean [ms]	1.27 ± 0.05[*]	1.86 ± 0.04	1.87 ± 0.04	2.30 ± 0.16[*]

^{*} p < 0.01 significant population difference between muscle/CSF and brain tissue (paired t-test).