17 O relaxation times in the rat brain at 16.4 tesla

Citation for published version (APA):

Document status and date:
Published: 01/05/2016

DOI:
10.1002/mrm.25814

Document Version:
Accepted author manuscript (Peer reviewed / editorial board version)

Please check the document version of this publication:

• A submitted manuscript is the version of the article upon submission and before peer-review. There can be important differences between the submitted version and the official published version of record. People interested in the research are advised to contact the author for the final version of the publication, or visit the DOI to the publisher’s website.
• The final author version and the galley proof are versions of the publication after peer review.
• The final published version features the final layout of the paper including the volume, issue and page numbers.
Link to publication

General rights
Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

• Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
• You may not further distribute the material or use it for any profit-making activity or commercial gain
• You may freely distribute the URL identifying the publication in the public portal.

If the publication is distributed under the terms of Article 25fa of the Dutch Copyright Act, indicated by the “Taverne” license above, please follow below link for the End User Agreement:
www.umlib.nl/taverne-license

Take down policy
If you believe that this document breaches copyright please contact us at:
repository@maastrichtuniversity.nl
providing details and we will investigate your claim.

Download date: 12 Sep. 2020
17O Relaxation Times in the Rat Brain at 16.4 Tesla

Hannes M. Wiesner,1,2 Dávid Z. Balla,1 G. Shajan,1 Klaus Scheffler,1,3 Kâmil Uğurbil,2† Wei Chen,2 Kâmil Uludag,4 and Rolf Pohmann1*†

Purpose: Measurement of the cerebral metabolic rate of oxygen (CMRO2) by means of 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 Tesla, 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 17O relaxometry at enriched concentrations after inhalation of 17O2 showed similar T2* values for gray matter (1.87 ± 0.04 ms) and white matter, significantly longer than muscle (1.27 ± 0.05 ms) and shorter than cerebrospinal fluid (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. Because 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.

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

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 [arteriovenous difference measurements (3–5) and positron emission tomography (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 intramolecular 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). Because 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 to assess the changes of the relaxation times with the temperature, which is critical due to the influence of viscosity and chemical exchange (19).

Previous relaxometric studies of H217O in rat brain have not differentiated between different tissue types in vivo. Finally, because pH, temperature, and concentrations of salts affect the chemical exchange and molecular motion, the linewidth of H217O is naturally autoregulated 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). E-mail: rolf.pohmann@tuebingen.mpg.de
Germany) based on a 16.4 Tesla (T) 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 µs (Resonance Research Inc., Billerica, MA). Custom-built 17O surface quadrature coils (diameter 1.5 cm; sensitive detection depth ~ 1 cm) (20) were tuned and matched for every sample individually to the 17O 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 better than -20 dB. For proton imaging, a separate 1H transmit and receive system was used instead. First and second order B0 shims were adjusted using FASTMAP (24) with a maximum sensitivity of the 17O-coil in Z-direction close to 1 T/m within a rise time of 212 µs.

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 h day/night cycle with free access to nutrition and water.

In Vivo Measurements

Ten male Wistar rats were used for in vivo relaxometry measurements (T1: n = 5; mean body weight 513 ± 71 g; T2: n = 5; mean body weight 529 ± 30 g). Anesthesia was induced in an induction chamber by 3–4% isoflurane (Forene®, Abbott, Chicago, IL). 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 17O-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) 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 min (21). For the maintenance of anesthetic efficacy during long experiments (22), the medetomidine infusion rate was increased every 75 min by 50 µg/kg/h.

Breathing rate and exhaled respiration gases (Capstar-100 End-tidal CO2 Analyzer, CWE Inc., Ardmore, PA) were monitored and recorded (PowerLab & LabChart 5; ADInstruments, Australia). Rectally measured body temperature was maintained at 36.8 ± 0.5°C by an electric heating pad connected through a radiofrequency (RF) filter. After the experiments, anesthesia was revoked using atipamezolhydrochlorid (Antisedan®, Pfizer Inc., New York, NY).

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 approximately 90 min 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 killed with >5% isoflurane without changing their position within the magnet. Acquisitions started 10 min 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 three-dimensional (3D) chemical shift imaging (CSI) sequence using weighted averaging of the k-space was applied (25,26). To obtain sufficient signal despite the short transverse relaxation time, the acquisition delay between the middle of the excitation pulse and the start of the acquisition was minimized to 484 µs. Because a nonselective excitation pulse was used, the sensitive region in the field of view (FOV) of the 3D CSI sequence with free induction decay (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 region of interest (ROI) in the center of the phantom was selected to avoid potential effects of superficial heating caused by 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°. A total of 10,240 FIDs for each inversion step were acquired within 7–15 min (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 nonlinear least-squares algorithms (Curve Fitting Toolbox, MATLAB®, The Mathworks Inc., Natick, MA). 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 signal-to-noise ratio (SNR) > 12 were fitted, which resulted in a ratio of 56% brain versus 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 T₂* at 17.6T) (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 h and 6 min 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 monoexponential 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 T₂*-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 h and 18 min. Postprocessing for T₂* was then performed as in vivo.

Relaxation times are given as mean ± 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 H₂¹⁷O water sample resulted in a T₁ value of 6.24 ± 0.37 ms over the sensitive region of the surface coil, indicating stable T₁-quantification and an efficient inversion over the entire FOV. In the same phantom, a T₂* of 2.24 ± 0.09 ms was found.

**In Vivo Relaxation Times T₁**

T₁-values for all rats are shown in Table 1. The T₁ 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 intracerebral contrast was observed at this resolution. A repeated measurement in the same rat in a different session demonstrated highly reproducible T₁-values (Table 1; rat 5).

**In Vivo and Postmortem Relaxation Times T₂***

In the high-resolution H₂¹⁷O images, intracortical 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. T₂* 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 cerebrospinal fluid (CSF), e.g., in the ventricles (in vivo: 2.21 ± 0.20 ms; postmortem: 2.30 ± 0.16 ms, Table 2). A T₂* 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

---

<table>
<thead>
<tr>
<th>In vivo T₁</th>
<th>Muscle tissue</th>
<th>Brain tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>rat 1 (583 g)</td>
<td>5.25 ± 1.02 (322 μL)*</td>
<td>6.34 ± 0.67 (477 μL)*</td>
</tr>
<tr>
<td>rat 2 (415 g)</td>
<td>6.47 ± 1.15 (459 μL)</td>
<td>6.84 ± 0.82 (441 μL)</td>
</tr>
<tr>
<td>rat 3 (483 g)</td>
<td>5.86 ± 1.10 (275 μL)*</td>
<td>7.64 ± 0.52 (282 μL)*</td>
</tr>
<tr>
<td>rat 4 (580 g)</td>
<td>5.22 ± 0.84 (214 μL)*</td>
<td>6.58 ± 0.39 (398 μL)*</td>
</tr>
<tr>
<td>rat 5 (504 g) #1</td>
<td>5.45 ± 0.71 (312 μL)*</td>
<td>6.79 ± 0.40 (398 μL)*</td>
</tr>
<tr>
<td>rat 5 (504 g) #2</td>
<td>5.74 ± 0.70 (312 μL)*</td>
<td>6.83 ± 0.45 (398 μL)*</td>
</tr>
<tr>
<td>Pop. Mean [ms]</td>
<td>5.67 ± 1.12*</td>
<td>6.84 ± 0.67*</td>
</tr>
</tbody>
</table>

*P < 0.05; significant population difference between muscle and brain tissue (paired t-test).
apparent in the signal time course of the localized FIDs as used for the T2* estimations (Fig. 3a). Extrapolation to TE = 0 yielded combined spin density- and T1-weighted images (Fig. 3c), showing highest signal intensity in muscle tissue and lowest in CSF and ventricles, confirming the lower T1 in muscle than in brain (Fig. 1c).

Rats undergoing 17O2-inhalations which were used for the postmortem T2* measurements showed a global increase of the image intensity by a factor of 4 to 5 compared with the natural abundance H217O signal, due to significant metabolic conversion of 17O2 to H217O. No intracortical 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 H217O concentration in equilibrium due to exhaustion of concentration gradients). Postmortem T2* values in muscle, GM and WM tissue were slightly increased compared with 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 H217O 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 17O2 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 17O.

**Phantom Studies**

Our phantom measurements are in relative agreement with results from previous nonlocalized measurements which reported values of T1 around 6–8 ms for enriched pure water (35) at physiological temperatures. Temperature controlled T2* measurements using continuous wave NMR reported T2* up to 4 ms at enriched H217O concentrations (36,37) in contrast to our significantly lower values, which could be a result of the higher achievable B0 field-homogeneity in nonlocalized NMR spectroscopy, of better temperature control in the nonlocalized measurements in NMR spectrometers and higher purity from paramagnetic ions of the glassware being

---

**Table 2**

In Vivo and Postmortem T2* Relaxation Times in ms of H217O measured in the Rat Head

<table>
<thead>
<tr>
<th></th>
<th>Muscle tissue</th>
<th>WM</th>
<th>GM</th>
<th>CSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>a) In vivo T2* (natural abundance)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rat A (567 g)</td>
<td>1.03 ± 0.18 (309 μL)</td>
<td>1.86 ± 0.15 (24 μL)</td>
<td>1.83 ± 0.13 (111 μL)</td>
<td>2.56 ± 0.23 (38 μL)</td>
</tr>
<tr>
<td>rat B (499 g)</td>
<td>1.07 ± 0.18 (296 μL)</td>
<td>1.78 ± 0.09 (29 μL)</td>
<td>1.75 ± 0.08 (128 μL)</td>
<td>2.19 ± 0.12 (27 μL)</td>
</tr>
<tr>
<td>rat C (503 g)</td>
<td>1.08 ± 0.17 (301 μL)</td>
<td>1.75 ± 0.11 (25 μL)</td>
<td>1.73 ± 0.10 (128 μL)</td>
<td>2.13 ± 0.16 (28 μL)</td>
</tr>
<tr>
<td>rat D (526 g)</td>
<td>1.15 ± 0.16 (361 μL)</td>
<td>1.71 ± 0.10 (20 μL)</td>
<td>1.77 ± 0.08 (148 μL)</td>
<td>2.14 ± 0.11 (27 μL)</td>
</tr>
<tr>
<td>rat E (552 g)</td>
<td>1.27 ± 0.15 (315 μL)</td>
<td>1.77 ± 0.08 (14 μL)</td>
<td>1.76 ± 0.15 (100 μL)</td>
<td>2.05 ± 0.11 (10 μL)</td>
</tr>
<tr>
<td>Pop. Mean [ms]</td>
<td>1.12 ± 0.09*</td>
<td>1.77 ± 0.06</td>
<td>1.77 ± 0.04</td>
<td>2.21 ± 0.20*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Muscle tissue</th>
<th>WM</th>
<th>GM</th>
<th>CSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>b) Postmortem T2* (enriched ~4–5 times of natural abundance)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rat F (250 g)</td>
<td>1.30 ± 0.21 (232 μL)</td>
<td>1.81 ± 0.17 (33 μL)</td>
<td>1.83 ± 0.15 (199 μL)</td>
<td>2.27 ± 0.35 (14 μL)</td>
</tr>
<tr>
<td>rat G (430 g)</td>
<td>1.28 ± 0.16 (239 μL)</td>
<td>1.84 ± 0.21 (25 μL)</td>
<td>1.85 ± 0.17 (182 μL)</td>
<td>2.26 ± 0.39 (9 μL)</td>
</tr>
<tr>
<td>rat H (540 g)</td>
<td>1.29 ± 0.25 (276 μL)</td>
<td>1.91 ± 0.33 (24 μL)</td>
<td>1.92 ± 0.21 (102 μL)</td>
<td>2.14 ± 0.44 (8 μL)</td>
</tr>
<tr>
<td>rat I (300 g)</td>
<td>1.20 ± 0.16 (207 μL)</td>
<td>1.87 ± 0.16 (22 μL)</td>
<td>1.89 ± 0.12 (175 μL)</td>
<td>2.53 ± 0.35 (25 μL)</td>
</tr>
<tr>
<td>Pop. Mean [ms]</td>
<td>1.27 ± 0.05*</td>
<td>1.86 ± 0.04</td>
<td>1.87 ± 0.04</td>
<td>2.30 ± 0.16*</td>
</tr>
</tbody>
</table>

*P < 0.01 significant population difference between muscle/CSF and brain tissue (paired t-test).
used (35). Furthermore, a fair amount of one-dimensional experiments in the 1970s accumulated in vitro estimations of muscle tissue T\textsubscript{1} and T\textsubscript{2} (37–39). In contrast to proton MRI, T\textsubscript{2}\textsuperscript{*} contrast changes close to air-sample borders were not observed in any images nor with varying voxel sizes as the frequency of H\textsubscript{2}\textsuperscript{17}O at

**FIG. 2.** Apparent in vivo transverse relaxation time T\textsubscript{2}*. a: Coronal FLASH proton image. b: CSI anatomical contrast of rat tissues in image intensity of H\textsubscript{2}\textsuperscript{17}O with intra-cortical differentiations of white matter and ventricles. c: T\textsubscript{2}\textsuperscript{*} estimates of H\textsubscript{2}\textsuperscript{17}O with pronounced differentiation between brain parenchyma (gray matter, white matter) and cerebrospinal fluid in the ventricles.

**FIG. 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\textsubscript{2}\textsuperscript{*} 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\textsubscript{2}\textsuperscript{17}O. c: T\textsubscript{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.
16.4 Tesla used in this study translates to a larmor frequency of 2.2T for protons. In addition, peak intensity changed by less than 15% comparing unlocalized spectra with and without B0-shimming, (i.e., with a smaller change in T2*).

Tissue Specific Relaxation and Anatomical Contrast

The differences between muscle and brain tissue H217O relaxation times have similar proportions as those known from proton measurements (40–42) and cannot be explained from temperature difference alone. However, intracortical differences are much less pronounced than for proton MRI due to the limited influence of the intermolecular environment on the field-independent relaxation of H217O (43). The lack of differentiable T2* relaxation times between gray and white matter and the clear separation by low intensity of white matter structures indicate a difference in either T1 or in H217O 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 H217O calibration (45).

The higher intersubject variability of the T1 measurements compared with the T2* data (Table 1 versus 2) is mainly due the lower sensitivity of the inversion recovery sequence compared with the simple FID acquisition used to determine T2*. In part, choosing larger voxels for the T1 measurements compensated for this. However, the low sensitivity of H217O in general requires multiple repetitions for averaging the signal both in T1 and T2* measurements, which is possible due to the generally short T1 in contrast to more slowly relaxing nuclei like protons.

Most previous investigations on oxygen consumption were limited in localization because of the low sensitivity of the 17O nucleus, thus neglecting potential contributions from nonbrain 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 T2* 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 postmortem 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 T2* 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 brain tissue $T_2^*$ values are remarkably close around 1.8 ms, because the tissue 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. Some of the most dominant factors for transverse H$_2$^{17}$O relaxation might represent a different mixture of tissue types.

**CONCLUSIONS**

The use of ultrahigh 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 $^{17}$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**

We thank Helmut Merkle and Michael Beyerlein for advice on RF-coils and animal setup. The study was funded by the Max Planck Society and supported in part by NIH grants.

**REFERENCES**