MRI Under Hyperbaric Air and Oxygen: Effects on Local Magnetic Field and Relaxation Times

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Purpose: Hyperbaric oxygen therapy has shown efficacies in the treatment of a number of diseases. The goal of this study was to develop a rodent hyperbaric chamber for MRI studies and to investigate the effects of hyperbaric air and hyperbaric oxygen on local magnetic field (B0) and MRI relaxation parameters in the rat brain.

Methods: A hyperbaric chamber, constructed to fit inside an animal MRI scanner, was pressurized with air to four atmospheres, while oxygen was delivered locally via nose cone. B0, T2, T2*, and T1 maps in the rat brain were evaluated under normobaric air, hyperbaric air, and hyperbaric oxygen at 7T.

Results: Under hyperbaric oxygen, images exhibited artifacts and temporal instability, attributable to fluctuating oxygen concentration from air and oxygen mixing near the imaging region. Physically shielding the imaging region from fluctuating oxygen concentration resolved the problems. With increasing oxygen at hyperbaric pressure, B0 was shifted downfield with increased inhomogeneity near the ear canals and nose. Brain T2 and T2* were lengthened, and T1 was shortened.

Conclusion: This study establishes the means to perform MRI on rodents under hyperbaric conditions. Hyperbaric air and hyperbaric oxygen have significant effects on B0 and tissue relaxation parameters compared with normobaric air. Magn Reson Med 000:000–000, 2013. © 2013 Wiley Periodicals, Inc.

Key words: oxygen therapy; BOLD; magnetic susceptibility; T1; T2; T2*; relaxation time constants

INTRODUCTION

Hyperbaric oxygen (HBO) therapy has been used to treat decompression sickness, air embolism, chronic wounds, stroke, traumatic brain injury, and cerebral palsy, among others (1). While the direct effect of HBO exposure is increased tissue oxygenation, a number of therapeutic effects have been proposed, including stimulating release of factors promoting healing, decreased intracranial pressure, and reduced cerebral edema (1). Prolonged HBO exposure is harmful, however. Noninvasive MRI could offer a means of studying physiological effects, oxygen toxicity, and to monitor treatment efficacy during HBO. Moreover, functional imaging studies under HBO where both arterial and venous blood are saturated with oxygen could provide insight into the role of oxygen on the regulation of cerebral blood flow, oxygen metabolism, and neurovascular coupling.

Paramagnetic molecular oxygen has several effects on MRI parameters, including the static magnetic field (B0), T2, T2*, and T1 (2–9). Ambient oxygen gas alters B0, which can lead to spatial shifts in MR images and fluctuations in signal intensity compared with air (2,3). Oxygen can also increase B0 inhomogeneity near air–tissue interfaces such as near the ear canal and nasal cavity, which can lead to susceptibility artifacts, such as signal dropout and geometric distortion in echo-planar imaging (2). Moreover, [O2] in the magnet bore could fluctuate when oxygen is delivered to the subjects, potentially affecting the temporal stability of MR signals.

Oxygen inhalation is known to increase blood T2 and T2* by increasing hemoglobin saturation, i.e., the blood oxygen level–dependent (BOLD) effect (5–7). Increased dissolved molecular oxygen in tissue and plasma water could also be expected to have a paramagnetic T2 and T2* shortening effect (2) but is normally masked by the BOLD effect. However, these paramagnetic effects of dissolved oxygen on T2 and T2* relaxation may not be negligible under HBO, where even venous hemoglobin is saturated with oxygen (10). BOLD functional MRI, which detects functional activation via changes in deoxyhemoglobin content, could thus give weaker responses under HBO. Similarly, oxygen inhalation decreases brain parenchyma T1 in the extravascular spaces as well as in blood plasma due to the paramagnetic dissolved oxygen (5,8,9,11). Such effects could complicate cerebral blood flow quantification using arterial spin labeling, in which blood and tissue T1 are important factors (9).

The goal of this study was to develop a rodent hyperbaric chamber for MRI studies and use it to investigate the effect of HBO on MRI relaxation properties and magnetic field. The challenges and solutions to designing the hyperbaric chamber are detailed. The hyperbaric chamber was used to investigate B0, T2, T2*, and T1 in the rat brain under normobaric air (NBAir), hyperbaric air (HBAir), and HBO conditions.
METHODS

Hyperbaric Chamber

A custom-made hyperbaric chamber for rodent MRI consisted of an animal cradle placed into a PVC pipe with O-rings on both ends of the cradle to provide a tight seal with the pipe (Fig. 1a). A threaded cap that screwed onto the pipe was used to hold the cradle in place in the pipe. The chamber was pressure-tested with water up to 5 atm absolute (ATA). Coaxial radiofrequency cables were connected through the endplate via SubMiniature version A (SMA) adapters. Lines of physiological monitoring equipment (temperature probe and pulse oximeter) were passed through a large hole in a bundle and sealed with silicone sealant or glue. Animal temperature was maintained with circulating warm water through a winding of rigid plastic tubing with the inlet and outlet passed through tight-fitting holes in one of the endplates.

Four gas lines, made of rigid plastic tubing, were pushed through very tight-fitting holes at the endplates. The chamber was pressurized with air to 4 ATA through one line with the input near one end. A second line on the opposite end of the chamber was open to the atmosphere outside the chamber to allow continuous airflow. A valve was put in the vent line outside the chamber to adjust the flow rate of the vent and thus the chamber pressure. A third gas line was used to deliver oxygen (or air) to the animal via a tightly fit nose cone with excess gas directed to the outflowing vent away from the head. The fraction of oxygen in the vented gas was maintained at <30% O₂ by adjusting flow rate of the pressurized air and the vent to avoid potential risk of explosion from concentrated pressurized oxygen in the chamber. Air was given through the nose cone when oxygen was not. The flow rate into the nose cone was set to 1.5 L/min. The fourth line was connected to a pressure meter to measure the chamber pressure.

In separate experiments outside MRI, a small tube was inserted to sample the gas in the outlet of the nose cone. The gas was sampled at low flow rates to minimize perturbing the venting of the chamber. The gas was directed to a small plastic bag at atmospheric pressure, and the percent oxygen in the sampled gas was measured (Surgi-Vet Capnograph, Smiths Medical PM Inc., Norwell, MA) after a steady reading was achieved over several minutes. We found that under our experimental HBO setup, the O₂ readings were 90%–93%.

Animal Preparation

The animal protocol was approved by the local Institutional Animal Care and Use Committee. Male Sprague-Dawley rats (n = 13, 254–600 g) were anesthetized with 1.5-g/kg urethane intraperitoneally and imaged under spontaneous breathing conditions. Respiration rate, heart rate, and arterial oxygen saturation were monitored (MouseOx, STARR Life Science Corp., Oakmont, Pennsylvania, USA), and rectal temperature was maintained at 37 ± 0.5°C with a feedback-regulated circulating warm water pad. Inhaled anesthetics, blood pressure, and blood–gas measurement were not attempted due to hyperbaric conditions. Animals were placed into a head holder with ear and tooth bars, into the cradle, and then into the
MRI Under Hyperbaric Conditions

FIG. 2. Temporal fluctuations of $^1$H$_2$O spectra during HBO in dead animals without (red dashed lines) and with the head cover and nose cone (solid black lines) to guard from oxygen fluctuations. a: Frequency resonance. b: Peak frequency. c: Peak intensity across time. The temporal fluctuations were eliminated with the head cover and nose cone. Under NBAir and HBAir spectra are similar to the "guarded from O$_2$ fluctuation" data.

hyperbaric chamber. Animals were imaged under NBAir, HBAir, and HBO conditions. Each trial of HBO exposure was $<25$ min to avoid possible oxygen toxicity effects (12) with similar periods of HBAir in between.

MRI

MRI was performed on a 7T magnet with 400 mT/m gradients (Bruker, Billerica, MA) and with a 2-cm transmit/receive surface coil. Global shimming was only performed once at the beginning of a session and was not repeated under different conditions. Nonlocalized spectroscopy was used to measure frequency shifts of the water peak using the following parameters: pulse repetition time (TR) = 300 ms; spectral width = 25 kHz; 6250 points zero-filled to 8192; and 160 repetitions. $B_0$ maps were acquired with a three-dimensional multiecho gradient echo sequence using the following parameters: field of view (FOV) = 25.6 $\times$ 25.6 $\times$ 30 mm$^3$; matrix = 64 $\times$ 64 $\times$ 75; TR = 20 ms; echo time (TE) = 2.3 and 8.1 ms; and three averages. $B_0$ was acquired once and spectroscopy was repeated 1–3 times for each of the NBAir, HBAir, and HBO conditions.

For $T_2$, multiecho fast spin echo was used with the following parameters: FOV = 25.6 $\times$ 25.6 mm$^2$; matrix = 96 $\times$ 96; seven 1.5-mm-thick slices; TR = 3 s; effective TE = 25, 40, 75, and 120 ms; echo train length = 8; and four averages. For $T_2^*$, multiecho gradient echo was used with the following parameters: FOV = 25.6 $\times$ 25.6 mm$^2$; matrix = 96 $\times$ 96; TR = 1.5 s; 10 TEs from 3.1 to 22.9 ms equally spaced by 2.2 ms; and two averages. Fourteen 0.75-mm-thick slices were acquired and consecutive pairs were averaged to give seven 1.5-mm-thick slices to minimize effects of through-plane dephasing. For $T_1$, inversion recovery gradient-echo planar imaging was used with the following parameters: FOV = 25.6 $\times$ 25.6 mm$^2$; matrix = 96 $\times$ 96; seven 1.5-mm-thick slices; TR = 12 s; TE = 9.9 ms; 10 inversion times (TI) from 23 to 3623 ms equally spaced by 400 ms; 2/3 partial Fourier acquisition; and three averages.

MRI Analysis

Image analysis was performed using MATLAB (MathWorks, Natick, Massachusetts, USA) and Statistical Parametric Mapping 5 (SPM5) software. $B_0$ maps in Hz were calculated with SPM5 from the image phase data. The realignment tool of SPM5 was used to measure the spatial shift in images due to hyperbaric conditions. $T_2$ maps were calculated using a linear fit of the logarithm of the data at different echo times to $\ln(M) = \ln(M_0) – (TE/T_2)$, where $M$ is the signal intensity at a given TE and $M_0$ is the equilibrium signal. $T_2^*$ maps were calculated similarly after averaging pairs of 0.75 mm slices to give 1.5-mm-thick slices. $T_1$ maps were calculated using a three-parameter nonlinear fit of the data at different inversion times to $M = M_0 – B M_0 e^{(-TI/T_1)}$, where $M$ is the signal intensity at a given TI, $M_0$ is the equilibrium signal, and $B$ is the efficiency of the inversion pulse.

Statistical Analysis

Group-average data are expressed as the mean $\pm$ standard error of the mean unless indicated otherwise. Relaxation times and frequency of the water peak were analyzed under NBAir, HBAir, and HBO conditions using analysis of variance followed by paired $t$ tests with Bonferroni-Holm correction for multiple comparisons. Corrected $P < 0.05$ was taken as statistically significant.

RESULTS

Minimizing Artifacts Under HBO

In our initial experience with MRI under HBO, most images exhibited artifacts. Moreover, the time-series images and the spectroscopic water frequencies and amplitudes were temporally unstable. Such instability was verified not to be physiological by repeating studies in phantoms and dead animals, which showed similar instability (Fig. 2, dotted red lines). Fluctuating [O$_2$] around the imaging region (i.e., the head) under HBO was suspected to be the cause of such instability. A solution was implemented by 1) covering the space around the head with a head cover made from hot melt adhesive to act as a barrier to the mixing of oxygen (from the nose cone) and air (from the end of the chamber) around the head, and 2) by tightly fitting the cone on the nose with excess gas diverted away from the animal toward the vent outlet (Fig. 1b,c). The MRI results with these solutions are also shown in Figure 2 (solid black lines). The instability was predominantly resolved. With the solutions
implemented, minor image artifacts and minor temporal instability under HBO, although tolerable, were observed occasionally in live animals but never in dead animals.

Effects of Hyperbaric Conditions on B₀
At steady state, the water spectroscopic frequencies under NBAir, HBAir, and HBO were all significantly different from each other (corrected \( P < 0.05 \)). The frequency differences between HBAir–NBAir and HBO–NBAir were \(-6.8 \pm 1.0\) and \(-28.5 \pm 6.3\) Hz, respectively (live animal, \( n = 4 \)). B₀ maps in Hz showed spatially heterogeneous frequency differences between conditions (Fig. 3). Between NBAir and HBAir, the largest differences were around the ear canals. Between HBAir and HBO, the largest difference occurred around the olfactory bulb and anterior brain structures due to the delivery of oxygen through the nose cone. B₀ changes due to oxygen resulted in spatial shifts in the images. The brain was shifted in the phase encode (dorsal–ventral) direction by \(-0.9–1.8\) pixels going from NBAir to HBAir and by \(<0.1\) up to 0.3 pixels from HBAir to HBO. Spatial shifts in the frequency encode (left–right) direction were negligible (<0.1 pixels). There were also subtle changes in shape and the extent of signal dropout of the brain going from normobaric air to hyperbaric conditions (data not shown).

Effects of Hyperbaric Conditions on Animal Physiology, \( T₂, T_{2}^*, \) and \( T₁ \)
Table 1 shows animal physiological parameters under NBAir, HBAir and HBO. Respiration and heart rates were reduced during HBAir and HBO compared with NBAir, but similar between HBAir and HBO. Whole brain \( T₂, T_{2}^*, \) and \( T₁ \) values during NBAir, HBAir, and HBO are shown in Fig. 4. \( T₂ \) and \( T_{2}^* \) were lengthened by HBAir and HBO compared with NBAir, while \( T₁ \) was shortened. In contrast to \( T₁ \) and \( T_{2}^* \), \( T₂ \) maps were not susceptible to fluctuating oxygen under HBO even without shielding. \( T₂ \) values were not significantly different between with and without shielding (\( P = 0.31, t \) test).

DISCUSSION
This study describes the construction of a hyperbaric chamber for rodent MRI under hyperbaric air and oxygen. HBO was achieved using air to pressurize the chamber with a separate line to deliver oxygen locally to the animal’s nose to prevent risks of explosion associated with highly concentrated oxygen. This implementation, however, leads to fluctuating oxygen levels around the imaged object, causing frequency and amplitude fluctuations, which result in image degradation and temporal instability. The solution was to shield the space around the imaged region and divert excess oxygen away from it. Such temporal instability was negligible under HBO.

With the solution as described, some minor artifacts during HBO exposure were still observed infrequently in \( T₁, T_{2}^*, \) and B₀ images in live animals but never in dead animals (\( n = 6 \) for \( T_{2}^* \) and \( n = 2 \) for \( T₁ \)). This observation suggests another source of artifacts in live animals, such as enhanced physiological noise. For example, respiratory noise from variation in magnetic susceptibility in the chest during the respiration (13) could be exacerbated by the high paramagnetic oxygen concentration in the lung under HBO given that HBAir data were free of artifacts. Physiological noise could be minimized by using gating, retrospective correction, or navigator eclipses in future studies (14,15). Additionally, fast spin echo acquisition can be used, as it was shown to be less susceptible to oxygen fluctuations, even without shielding.

Effects on B₀
Under steady-state hyperbaric conditions, the increased ambient oxygen shifted the water frequency, resulting in a position shift relative to NBAir, which can be readily corrected by image coregistration. Hyperbaric conditions also created magnetic field inhomogeneity in the nose region and ear canal because of the ambient oxygen around these air–tissue interfaces. From NBAir to HBAir, magnetic field inhomogeneity increased in the ear canal as expected. Similar shielding around the ear or using filler (such as toothpaste) in the ear canal can also be

<table>
<thead>
<tr>
<th>Physiological Parameters in Animals</th>
<th>NBAir</th>
<th>HBAir</th>
<th>HBO</th>
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<tbody>
<tr>
<td>Respiration rate in breaths/min</td>
<td>121 ± 14 (6)</td>
<td>101 ± 19* (4)</td>
<td>115 ± 4 (3)</td>
</tr>
<tr>
<td>Heart rate in beats/min</td>
<td>387 ± 37 (10)</td>
<td>360 ± 28* (10)</td>
<td>350 ± 22* (9)</td>
</tr>
<tr>
<td>SpO₂ in %</td>
<td>95.0 ± 1.9 (10)</td>
<td>98.4 ± 0.5* (10)</td>
<td>98.4 ± 0.5* (9)</td>
</tr>
</tbody>
</table>

Data are presented as the mean ± standard deviation (n). The smaller sample sizes for HBAir and HBO are due to loss of the respiration signal from the oximeter.

\* \( P < 0.05 \) versus NBAir (paired t test).
applied to the ear regions to avoid field inhomogeneity. From HBAir to HBO, magnetic field inhomogeneity increased in the nose regions, affecting the olfactory bulb and anterior brain. B₀ field maps could be used to correct the field inhomogeneity. The effect of ambient oxygen on B₀ has been shown to have undesirable consequences in various MR applications, such as altering image intensity in BOLD functional MRI of hyperoxic inhalation (2,3) or altering phase in MR thermometry (4) and phase-contrast MR angiography.

Effects on T₂, T₂*, and T₁

Compared with normobaric air, air at 4 ATA would have 4 times the oxygen, normobaric oxygen (NBO) would have 4.76 times the oxygen, and pure oxygen at 4 ATA would be expected to have 19 times the oxygen content. We measured the percent O₂ in the outlet of the nose cone to be around >90%, indicating our experimental conditions were close to achieving the ideal of 100% oxygen at hyperbaric pressure. Because HBAir at 4 ATA has similar [O₂] as NBO, we expect these conditions would have similar effects on relaxation parameters. However, whole brain T₂ increased by 1% during HBAir compared with NBAir, which was smaller than previously reported T₂ increases of 4%–16% in mice at 9.4T (5) and 3%–12% in rats at 7T (6) during NBO compared with NBAir. Whole brain T₂ increased by only 2% during HBO compared with NBAir. Similarly, whole brain T₂* increased during HBAir by 7% and during HBO by 8%, values that are also smaller than a previous study reporting a brain T₂* increase of 13%–23% in rats at 7T during NBO compared with NBAir (6). Experimental conditions such as field strength, species, anesthesia [pentobarbital/urethane (5) or chloral hydrate (6)], and whether animals were mechanically ventilated (6) or not (5) do not appear to have noticeable effects on the T₂ changes. Additionally, the regions of interest analyzed may affect the results, with large differences between ROIs within the same studies (5,6).

In addition to affecting T₂ and T₂* through hemoglobin, dissolved paramagnetic oxygen in the blood and tissue should also have a direct T₂ and T₂* shortening effect (2). Under normobaric air conditions, the BOLD effect from deoxyhemoglobin is dominant (5,6). With sufficiently high dissolved blood oxygen when hemoglobin is saturated, the BOLD effect may also become saturated, and the relaxation enhancement effect could begin to counteract it. Venous hemoglobin saturation is only 87% (pO₂ = 57 mmHg) under NBO but is essentially 100% (pO₂ = 424 mmHg) under HBO at 3 ATA (10). The changes in T₂ and T₂* herein tended to be smaller going from HBAir to HBO than from NBAir to HBAir, suggesting the BOLD effect was possibly saturated. Functional MRI BOLD contrast could thus be reduced at HBO.

Whole brain T₁ was shortened during HBAir by 2% compared with NBAir, which is similar to or smaller than some previously reported T₁ decreases of 4%–11% in mice at 9.4T (5), 3%–7% in rats at 7T (6), and 2% in rats at 7T (8) during NBO compared with NBAir. Whole brain T₁ was shortened during HBO by 7% compared with NBAir, substantially smaller than expected based on the assumed [O₂] differences between NBAir and HBO. HBO-induced T₁ shortening could have implications in blood flow quantification using arterial spin labeling MRI (9). Arterial pO₂ under HBO is >2000 mmHg (10,16,17), which would alter arterial T₁ significantly, in turn substantially increasing the rate at which the label signal decays in blood and increasing the difference between arterial T₁ and tissue T₁, confounding blood flow quantification by arterial spin labeling (18–20). Blood T₁ and cerebral blood flow measurements under HBO are currently under investigation. T₁ measurement has been used to evaluate oxygen tension in body fluids such as cerebrospinal fluid and vitreous under normobaric conditions (11,21), and it could also be applied to hyperbaric conditions.

CONCLUSIONS

This study described the design of a rodent hyperbaric chamber for MRI studies and demonstrates the feasibility of performing MRI during hyperbaric air and oxygen. Under hyperbaric oxygen conditions, there were image artifacts from the rapid fluctuations in oxygen levels due to mixing of air and oxygen at hyperbaric pressures.

FIG. 4. Whole brain T₂, T₂*, and T₁ during NBAir, HBAir, and HBO (mean ± standard error of the mean; n shown at the bottom of each bar). Significant differences are indicated by the brackets with given uncorrected P values. T₁ and T₂* data acquired during HBO without shielding were excluded from analysis due to artifacts. Furthermore, T₂* HBO data were discarded in one animal with shielding in which the T₂*-weighted images still had significant artifacts.
Solutions were implemented to minimize image artifacts. Hyperbaric air and hyperbaric oxygen showed significant effects on $B_0$ and tissue relaxation parameters compared with normobaric air.

REFERENCES