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BRIEF COMMUNICATION

Non-invasive measurement of cerebral oxygen metabolism in the mouse brain by ultra-high field ^{17}O MR spectroscopy

Weina Cui[1], Xiao-Hong Zhu[1], Manda L Vollmers[1], Emily T Colonna[1], Gregor Adriany[1], Brandon Tramm[2], Janet M Dubinsky[3] and Gülin Öz[1]

To assess cerebral energetics in transgenic mouse models of neurologic disease, a robust, efficient, and practical method for quantification of cerebral oxygen consumption is needed. ^{17}O magnetic resonance spectroscopy (MRS) has been validated to measure cerebral metabolic rate of oxygen ($CMRO_2$) in the rat brain; however, mice present unique challenges because of their small size. We show that $CMRO_2$ measurements with ^{17}O MRS in the mouse brain are highly reproducible using 16.4 Tesla and a newly designed oxygen delivery system. The method can be utilized to measure mitochondrial function in mice quickly and repeatedly, without oral intubation, and has numerous potential applications to study cerebral energetics.

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INTRODUCTION

Despite its relatively small size (~2% of body weight), the brain consumes ~20% of all oxygen used in the body. Impairments in the ability of mitochondria to fuel brain function are implicated in many neurodegenerative diseases.[1] However, *in vivo* confirmation of mitochondrial dysfunction in these diseases is largely lacking.

Techniques to assess cerebral energetics *in vivo* include ^{13}C magnetic resonance spectroscopy (MRS),[2] ^{31}P MRS,[3] and positron emission tomography.[4] ^{13}C magnetic resonance spectroscopy bears substantial challenges for studies in the mouse, including the need to place arterial and venous catheters, which precludes longitudinal measurements, and the small blood volume of mice, which precludes blood draws in the scanner (needed for proper analysis of the data). ^{31}P magnetization transfer MRS yields ATP metabolism rates, however, requires long data acquisition times in the mouse brain because of low sensitivity. ^{18}F-fluorodeoxyglucose positron emission tomography imaging can assess hypometabolism in neurologic diseases,[4] however, involves repeated radiation exposure in longitudinal studies. ^{15}O positron emission tomography has been used to image cerebral oxygen utilization rate in the human brain;[5] however, it is difficult to map cerebral metabolic rate of oxygen ($CMRO_2$) in mouse brain by this method because of limited spatial resolution. In addition, the ^{15}O radioactive tracer has very short half-life (~2 minutes), thus, requires on-site tracer generation. Therefore, a robust and practical method that can directly quantify cerebral oxygen consumption and that can be applied repeatedly in transgenic mouse models of neurologic disease is highly needed to address the involvement of cerebral energetics in these diseases.

^{17}O MRS imaging (MRSI) can be utilized to map $CMRO_2$,[6] and has been rigorously validated in rat brains[7] and applied to small animal models including healthy cats[8] and ischemic mice.[9] ^{17}O is a stable, MR-active isotope of oxygen. Despite its low isotopic enrichment (0.037%), the natural abundance ^{17}O-water ($H_2^{17}O$) can be detected quickly with high sensitivity because the concentration of $H_2^{17}O$ at natural abundance is ~20mmol/L. Cerebral metabolic rate of oxygen is measured from an increase in the $H_2^{17}O$ signal above natural abundance that results from mitochondrial metabolism of $^{17}O_2$ gas inhaled over 2 to 3 minutes.[6] Thereby the methodology produces three-dimensional maps of $CMRO_2$ quickly and reliably. However, mice present unique challenges for ^{17}O MRSI because of their small size. First, oral intubation, which has been essential for controlled ^{17}O gas delivery in rats and cats, is substantially more challenging in mice. Mouse intubations are feasible,[10] however, require extensive training and practice, making the measurements dependent on an individual skilled in this procedure and preventing practical and routine applicability of the method. Second, minimization of the dead space between the ^{17}O reservoir and the mouse is required for rapid gas switching and to limit the use of the expensive $^{17}O_2$ gas (currently ~$3,000 for 1 L of 70% enriched gas). One study demonstrated the feasibility of ^{17}O MRSI at 11.7T in stroke mice,[9] where each mouse serves as their own control and $CMRO_2$ can be compared bilaterally. Critically, the detection sensitivity for the $H_2^{17}O$ signal increases in a supra-linear fashion with increasing magnetic field.[6,11] Here, our goal was to develop robust, efficient, and practical methodology to measure regional $CMRO_2$ in the mouse brain at 16.4T and to assess the reproducibility of the measurements.

[1]Center for MR Research, Department of Radiology, Medical School, University of Minnesota, Minneapolis, Minnesota, USA; [2]Virtumed, LLC, University of Minnesota, Minneapolis, Minnesota, USA and [3]Department of Neuroscience, University of Minnesota, Minneapolis, Minnesota, USA. Correspondence: Dr G Öz, Center for MR Research, 2021 6th Street S.E., Minneapolis, MN 55455, USA.
E-mail: gulin@cmrr.umn.edu
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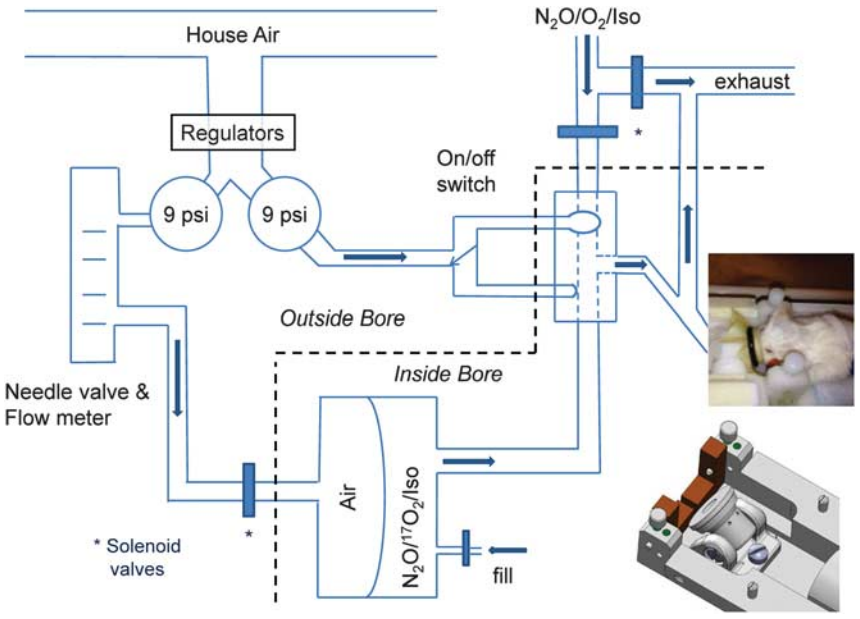

Figure 1. Gas delivery system. A pneumatically remotely controlled balloon switch determines which gas reaches the mouse: the isoflurane and normal O_2/N_2O mixture or a comparable mixture with $^{17}O_2$ from a gas-tight two-chamber reservoir placed in front of the mouse inside the bore. Solenoid valves control delivery of air pressure to release the $^{17}O_2$ over 2 to 3 minutes. The tightly closed mouse nose cone (designed to provide flexibility in the head angle, shown in the inset) prevents leaking of $^{17}O_2$.

MATERIALS AND METHODS

Subjects

All animal experiments were approved by the University of Minnesota Institutional Animal Care and Use Committee and performed according to the National Research Council's Guide for the Care and Use of Laboratory Animals. Five healthy male mice on FVB ($N = 2$, age 20 to 25 weeks; weight 35 to 40 *g*) and C57B6/CBA ($N = 3$, 7 to 10 weeks; 27 to 30 *g*) backgrounds were used.

Animal Preparation

Animals were induced with 3% to 4% isoflurane and a 1:1 mixture of O_2:N_2O. Spontaneously breathing mice were fixed in a custom built mouse holder/nose cone system and maintained anesthetized with 1.2% to 2% isoflurane while monitoring body temperature and respiration rate (SA Instruments, Stony Brook, NY, USA) during MR scanning. Body temperature was maintained at $\sim 37^{\circ}C$ with a circulating warm water system and a heating fan system.

In addition, a MouseOx Plus (Starr Life Sciences, Oakmont, PA, USA) system was used to monitor heart rate, oxygen saturation, breathing rate, and pulse distension when testing the ^{17}O gas delivery system outside the scanner.

Gas Delivery System

A custom, switchable, magnet-compatible gas delivery system was developed to provide $^{17}O_2$ to the anesthetized mouse (Figure 1). The mouse's nose was held in place through a flexible membrane into a conical nose cone by an o-ring. The nose cone had an exhaust port vented to the outside and an input port connected to the common port of a manually controlled three-way, pneumatic balloon valve (Hans Rudolph, Shawnee, KS, USA). Pressure (9 psi) for the balloon valve was stepped down from house air. The normally open balloon valve port provided N_2O/O_2/isoflurane gas from a mixing valve and vaporizer to the nose cone. The $N_2O/^{17}O_2$/isoflurane gas mixture was loaded into one side of a custom designed non-diffusible, flexible 250 mL double compartment gas reservoir (Hans Rudolph) and the composition of the gas mixture was ascertained with a capnometer (Capnomac Ultima, *ULT-SVI*, Datex, Helsinki, Finland). The output port of the reservoir was connected to the alternate input port of the balloon valve. To expel the ^{17}O gas mixture, the back side of the gas reservoir was filled with air. Programmable solenoid valves (ValveBank8, Automate Scientific, Berkeley, CA, USA) gated the flow of low pressure air (9 psi) to fill the back reservoir and vent the normal O_2 mixture to the exhaust stream. Timing of the manually switched balloon valve was also triggered by the ValveBank8. The balloon valve and the gas reservoir were positioned as close to the mouse as possible to minimize dead space and speed the delivery of the $^{17}O_2$ gas mixture. The balloon valve was positioned inside the magnet bore with the pneumatic switch extending into the surrounding control room. The gas reservoir was kept inside the scanner bore or positioned just outside the bore if refilling was needed during the scanning session.

Magnetic Resonance Protocol

All MRI studies were performed using a 16.4 T Varian/Magnex (26 cm bore) system. A home built linear surface ^{17}O coil (14mm diameter, 94.6 MHz) combined with a quadrature ^{1}H coil (16 mm diameter, 700 MHz) designed for minimal cross-talk was used to acquire anatomic ^{1}H images and ^{17}O MRSI data. Axial anatomic ^{1}H images were acquired using a fast spin echo imaging sequence (repetition time TR = 4 seconds, echo spacing = 12 milliseconds, echo train length = 16, echo time TE = 48 milliseconds, field-of-view = 20 mm × 20 mm, matrix size = 256 × 256, slice thickness = 2.4 mm, averages = 2, total acquisition time = 4 minutes). Three-dimensional ^{17}O MRSI data were acquired using a previously described Fourier Series Window MRSI technique[9] in which the k-space sampling is weighted according to the Fourier coefficients of a predetermined cylindrical voxel with voxel size of 26 μL (9.3 μL nominal). Acquisition parameters for the ^{17}O MRSI data were: *TR/TE* = 10 milliseconds/0.35 milliseconds, phase encodes = 9 × 9 × 5, *FOV* = 20 mm × 20 mm × 12 mm, spectral width = 20 kHz, number of slices = 5, slice thickness = 2.4 mm, total scan number = 1,542 and hence, acquisition time = 15.4 seconds per 3D-MRSI volume. The free-induction-decays were zero-filled, and a line broadening of 100 Hz was applied before fast Fourier transformation.

For $CMRO_2$ measurements, the $^{17}O_2$ gas (70% enriched, Cambridge Isotope Laboratories, Tewksbury, MA, USA) was mixed with N_2O gas ($\sim$1:1) and isoflurane, and stored in the custom designed gas reservoir. After ^{17}O MRSI data were acquired for 3 minutes during non-labeled O_2 inhalation, the respiration gas was switched to the $^{17}O_2$ mixture while ^{17}O MRSI data were continuously acquired. After $\sim$2.5 min of $^{17}O_2$ inhalation, the gas was switched back to the unlabeled O_2/N_2O gas mixture, and the ^{17}O MRSI acquisition was continued for another 15 minutes. Each mouse underwent a second $^{17}O_2$ inhalation period (2.5 min) 30 minutes after the completion of the first $^{17}O_2$ inhalation.

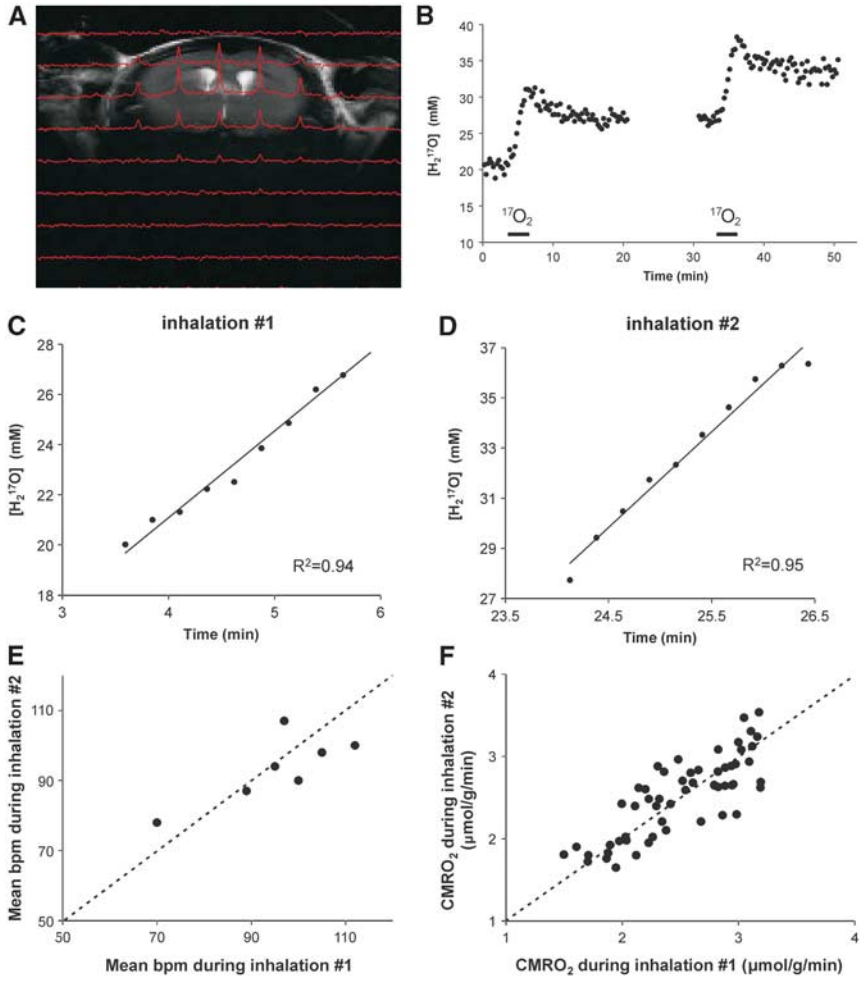

Figure 2. Determination of $CMRO_2$ in mouse brain at 16.4T using ^{17}O magnetic resonance spectroscopy imaging (MRSI). (**A**) ^{1}H T_2-weighted coronal anatomic image overlaid with the corresponding slice of the ^{17}O three-dimensional (3D) MRSI image of $H_2^{17}O$ captured 1.5 minutes into an inhalation in the mouse brain at 16.4T. The signal-to-noise ratio of $H_2^{17}O$ was increased ~40% compared with the natural abundance brain $H_2^{17}O$ signal at this point. The spectral time course has a temporal resolution of 15.4 seconds. Each 3D-MRSI ^{17}O image has a nominal resolution of 9.3 μL. (**B**) The time course of the $H_2^{17}O$ signal obtained from a striatal voxel before, during, and after two brief $^{17}O_2$ inhalations (bars). (**C**, **D**) Slopes of the linear fits to each of the $^{17}O_2$ inhalations yields the cerebral metabolic rate of oxygen ($CMRO_2$). (**E**) Physiologic conditions (b.p.m. = breaths per minute) were stable during both inhalations. (**F**) Within-session test–retest reproducibility of $CMRO_2$ measurements as shown by the relationship between two $^{17}O_2$ inhalations across multiple voxels ($N = 55$). The identity line is also shown in panels **E** and **F**.

Cerebral Metabolic Rate of Oxygen Calculation

The $H_2^{17}O$ resonance intensities from each voxel were converted to absolute $H_2^{17}O$ concentrations using the natural abundance signal (20.35 μmol/g) from the same voxel as an internal reference. Linear regression of the brain $H_2^{17}O$ concentration time courses during the $^{17}O_2$ inhalation period was applied and the slopes were used for calculating the $CMRO_2$ values. This simplified model has been previously validated in rat brains with short $^{17}O_2$ inhalation where fast exchange of the oxygen gas is expected.[7,12] Similarly, this completely non-invasive $CMRO_2$ quantification method was applied to mouse model in the present study.

RESULTS

The newly designed gas delivery system (Figure 1) was tested on the bench and inside the scanner to ensure that there were no gas leaks. No changes were observed in the mouse heart rate, oxygen saturation, breathing rate, and pulse distension during the switch to the ^{17}O gas reservoir. The system ensured efficient gas delivery, with ~100 to 120 mL $^{17}O_2$ (70% enriched) required per 2.5-minute inhalation period with enriched gas.

For $CMRO_2$ measurements, the $^{17}O/^{1}H$ coil was set up such that oxygen utilization could be measured with high sensitivity in a volume encompassing the striatum, a structure of high interest for various neurodegenerative diseases. At a nominal voxel size of 9.3 mm^3 (diameter of 2.2 mm and height of 2.4 mm for the cylindrical voxel) and a temporal resolution of 15.4 seconds, the signal-to-noise ratio of the ^{17}O MRSI data were sufficient for reliable $CMRO_2$ measurements in the sensitive area of the coil (Figure 2A).

The feasibility of measuring $CMRO_2$ in healthy mice was demonstrated with 2.5-minute inhalations of $^{17}O_2$. The time course of the $H_2^{17}O$ concentration obtained from a striatal voxel demonstrates the incorporation of inhaled $^{17}O_2$ into ^{17}O-water via mitochondrial metabolism (Figure 2B). The oxygen consumption rate was calculated from linear fits to the slopes of rising $H_2^{17}O$ levels during the inhalations (Figures 2C and 2D). An average striatal oxygen consumption rate of 2.6 ± 0.4 (SD) μmol/g per minute ($N = 28$) was calculated from 14 bilateral voxels of five healthy mice (two scanned twice). Mice were stable physiologically during all sessions, as illustrated by their spontaneous

breathing rates (Figure 2E). Isoflurane levels (1.6 ± 0.2%, mean ± s.d.) and body temperature (37.0 ± 0.4°C, mean ± s.d.) during the $^{17}O_2$ inhalations did not vary appreciably between animals. Repeat inhalations demonstrated excellent within-session reproducibility with a mean test–retest coefficient of variance (*CV* = s.d./mean) of 6.5% for $CMRO_2$ from all voxels in the sensitive area of the coil (Figure 2F) and a *CV* of 5% from the striatal voxel.

DISCUSSION

Here we demonstrated that efficient and practical ^{17}O MRS measurements of $CMRO_2$ in the mouse brain are feasible with high sensitivity and reproducibility using 16.4T, a surface $^{1}H/^{17}O$ coil and a newly designed gas delivery system. The method has numerous potential applications to mouse models of human diseases because there is substantial interest in 'tweaking' energy metabolism to prevent and treat neurologic disorders[13] and because such treatments are almost always tested in mouse models first.[14,15]

The dynamic change of the $H_2^{17}O$ signals observed in the mouse brain during and after the $^{17}O_2$ inhalation was similar to previous observations in the rat and cat brains[7,8] showing the accumulation of the metabolic $H_2^{17}O$ during the $^{17}O_2$ inhalation and the $H_2^{17}O$ signal decay after switching back to non-labeled oxygen gas. Namely, the cerebral $H_2^{17}O$ concentration reached a new steady state level at the end of the $CMRO_2$ measurement that was higher than the natural abundance or baseline $H_2^{17}O$ level because of the introduction of the ^{17}O label.

Although one prior study demonstrated the feasibility of $CMRO_2$ measurements by ^{17}O MRSI at high field,[9] the issue of efficient gas delivery was not addressed previously. The current study aimed to move the methodology to routine use by optimizing efficient delivery of the expensive $^{17}O_2$ gas. Namely, normal tidal volumes are ~0.25 mL in mice. The 200 to 250 mL of $N_2O/^{17}O_2$/isoflurane gas mixture flowing during a 2.5-min inhalation provides sufficient airflow to sustain normal respiration without compromising breathing rates or producing rebreathing and results in a per inhalation cost of ~$350 for ^{17}O gas. In addition, a higher field scanner was utilized in the current study relative to the prior study,[9] enabling a higher spatial resolution (9.3 versus 15 μL nominal), which was sufficient to localize, e.g. the striatum. Note, however, that this was not a strict comparison of the ^{17}O sensitivities at the two fields, which is best accomplished by utilizing the same set-up (coil/phantom/animal) at both fields.[11]

In this pilot study, we utilized two different background strains and mice at different ages to make sure that the O_2 delivery system worked with different sized mice and to include any biological variability due to these variables within the measurements. To tease out specific effects of age and strain, a larger and more systematic study should be performed.

The mean striatal $CMRO_2$ obtained here was higher than previously reported $CMRO_2$ in anesthetized rat brain,[7] which is expected, as metabolic rates are higher in mice than rats. In addition, the oxygen utilization rate obtained here was consistent with previously reported $CMRO_2$ in anesthetized mouse brain.[9] Finally, the excellent reproducibility demonstrated in this study (5% to 7% within-session test–retest *CV*) indicates that this method can be applied in the future to test responses to physiologic challenges in individual mice.

DISCLOSURE/CONFLICT OF INTEREST

The authors declare no conflict of interest.

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