Non-Invasive measurement of the cerebral metabolic rate of oxygen using MRI in rodents [version 1; peer review: 1 approved, 1 approved with reservations]

Tobias C Wood, Diana Cash, Eilidh MacNicol, Camilla Simmons, Eugene Kim, David J Lythgoe, Fernando Zelaya, Federico Turkheimer

Department of Neuroimaging, Institute of Psychiatry, Psychology & Neuroscience, King's College London, SE5 8AF, UK

Abstract
Malfunctions of oxygen metabolism are suspected to play a key role in a number of neurological and psychiatric disorders, but this hypothesis cannot be properly investigated without an in-vivo non-invasive measurement of brain oxygen consumption. We present a new way to measure the Cerebral Metabolic Rate of Oxygen (CMRO\textsubscript{2}) by combining two existing magnetic resonance imaging techniques, namely arterial spin-labelling and oxygen extraction fraction mapping. This method was validated by imaging rats under different anaesthetic regimes and was strongly correlated to glucose consumption measured by autoradiography.

Keywords
MRI, CMRO\textsubscript{2}, CBF, OEF, Glucose, Metabolism
Introduction
The brain requires around 20% of a human’s energy production, and hence requires a similar proportion of the body’s oxygen supply. There is great interest in being able to quantitatively map the Cerebral Metabolic Rate of Oxygen (CMRO₂) consumption, both as a marker of pathology and for the study of healthy ageing. Although methods exist using oxygen isotopes with either Magnetic Resonance (MR) spectroscopic imaging or Positron Emission Tomography (PET), it would be advantageous to use proton-based Magnetic Resonance Imaging (MRI) methods due to their low invasiveness, lower cost, and wider availability. Recent years have seen the emergence of methods including whole-brain measurements of CMRO₂ using a combination of T2-mapping and phase-contrast velocity measurements, voxel-wise mapping using quantitative Blood Oxygenation Level Dependent (qBOLD) with calibration from gas administration and high-resolution mapping methods based on Quantitative Susceptibility Mapping (QSM).

For this study we created a straightforward and robust method to measure CMRO₂ by combining measurements of Cerebral Blood Flow (CBF) and Oxygen Extraction Fraction (OEF) made with a pre-clinical MRI scanner. We calculated CBF maps using Arterial Spin Labelling (ASL) and OEF maps were constructed by measuring the reversible rate of transverse relaxation $R'_2$, which is related to the concentration of deoxyhaemoglobin (dHb), and DBV, which is defined as the difference between $R'_2$ and the irreversible relaxation rate $R_2$, where relaxation rates are the inverses of relaxation times ($T'_2 = 1/R'_2$). MR images can be acquired with $T'_2$-weighting using an Asymmetric Spin-Echo (ASE) sequence where the refocusing pulse is offset from the standard time to produce a spin echo, $T/2$, by an echo-shift $\tau/2$, which can be either positive (the pulse occurs later than $T/2$) or negative (the pulse occurs earlier than $T/2$). Echoes formed at the same $T_\phi$ but different $\tau$ will hence have the same $T'_2$-weighting, but different amounts of additional $T'_2$ (or $R'_2$) weighting. By observing the signal in each voxel from multiple $\tau$ values, we can measure a mono-exponential $R'_2$ as we would measure $R_2$ from multiple values of $T_\phi$.

However, in brain tissue the observed signal value at $\tau = 0$ is less than would be expected from extrapolating the signal curve for $\tau \neq 0$ back to the origin. This discrepancy can be attributed to static dephasing of spins in susceptibility gradients. The principle biological contributor to such gradients is the presence of deoxyhaemoglobin (dHb) in draining veins. In this model, the dephasing only affects the signal evolution below the critical time $\tau = T_c$. The signal $S$ can hence be described in two parts:

$$S(\tau) = \begin{cases} S_0 \exp \left( \frac{-0.3(R'_2 \tau)^2}{DBV} \right) & \tau < T_c \\ S_0 \exp \left( -R'_2 \tau + DBV \right) & \tau > T_c \end{cases}$$

These equations are equivalent to those used by Stone & Blockley, but formulated purely in terms of $R'_2$ and Deoxygenated Blood Volume (DBV) and we have neglected the dependence of $S_0$ on TE and $T'_2$ for clarity. The OEF can then be found by

$$OEF = \frac{3R'_2}{4\pi DBV \gamma B_0 \delta \chi_0 Hct}$$

where $\gamma = 2\pi \times 42.577$ MHz is the proton gyro-magnetic ratio, $B_0$ is the magnetic field strength, $\delta \chi_0 = 0.264 \times 10^{-6}$ is the susceptibility difference between oxygenated and deoxygenated blood cells, and haematocrit (Hct) is related to haemoglobin concentration by Hct = 0.03[HB]. In previous clinical studies it has been possible to estimate DBV from the ASE data. We found that we could not reliably fit the data for both DBV and OEF at 9.4T and hence assumed a fixed value of 3.3% for DBV.

$R'_2$ is not only affected by deoxygenated blood, but by any source of susceptibility gradients. The principal of these are
background or Macroscopic Field Gradients (MFGs) from air/tissue interfaces, which can be corrected with Z-shimming \cite{8,19}. A Z-shim is an additional small gradient played during the spin-echo formation which partially rephases signals voxels affected by MFGs, but de-phases signal in unaffected voxels \cite{22,23}. By acquiring and combining multiple images with different Z-shims, the lost signal from MFGs can be restored across the whole image, but will not affect the signal from sub-voxel susceptibility gradients due to deoxygenated blood \cite{16}. In the human brain the largest MFGs are present above the nasal sinuses, where air is closest to the parenchyma, and hence the largest susceptibility gradient exists in the Z (axial, in humans superior-inferior) direction. In rodents, the largest voids within the head are the mastoids, and in addition the skull and the tissue surrounding the brain are significantly thinner than in humans. We hence found that gradients in the Y (in animals the superior-inferior) direction were also a significant issue and so added shimming in both the Z and Y directions.

**Imaging protocol**

A total of ten adult male healthy Sprague-Dawley rats (440–537 g; Charles River) were imaged in a 9.4 Tesla pre-clinical MR system using a four-channel head receive coil, transmit body coil and separate ASL labelling coil (Bruker GmbH). All rats were initially anaesthetised with 5% isoflurane in an 80:20 mix of air and medical oxygen. Five of the rats were maintained with 2.5% isoflurane for the duration of scanning, while the remaining five received a bolus of 65 mg kg⁻¹ alpha-Chloralose (α-Chloralose) solution in saline, administered through a tail vein cannula, followed by continuous infusion at a rate of 30 mg “kg”⁻¹“h”⁻¹.

All animals were scanned with the same protocol consisting of MP2-RAGE \cite{34}, ASL, and ASE images. The MP2-RAGE structural T1-weighted image was acquired with a matrix size of 160x160x128, isotropic 0.19mm voxel size, TE/T1/T2/TR = 2.7/900/3500/9000 ms, and flip-angles $\alpha_1/\alpha_2 = 7/9°$. An additional Ultrashort Echo Time (UTE) COMPOSER scan was acquired for coil combination \cite{35}.

For ASL we used the manufacturer’s Continuous ASL (CASL) sequence with a spin-echo Echo Planar Imaging (EPI) readout \cite{36}. The matrix size was 96x96 with 18 axial (rostro-caudal) slices, 0.26x0.26x1.5 mm voxel size, TE/TR = 13.5/4000 ms, partial-fourier 66%, label time 3000 ms, post-label time 300 ms \cite{36,27}, and 30 pairs of label/control images, scan time 4 minutes. The labelling plane was positioned 5 mm behind the carotid artery split, which was found using a localizer scan acquired with the labelling coil as per the manufacturer’s instructions. Two single-volume reference scans were acquired using the same sequence settings and no labelling power, one of which had reversed phase encode direction (see below).

For the ASE sequence we modified the manufacturer’s spin-echo EPI sequence to allow the 180° refocusing pulse to be offset by $\tau$ as defined above. The matrix size and resolution were matched to the ASL sequence, but with TE/TR = 70/1800 ms. Partial Fourier was switched off to minimise any intensity modulation from the echo moving out of the acquisition window in the readout (X, left-right) direction \cite{39}. Twelve values of $\tau$ spaced from -32 to 56 ms were acquired. At each, five Z-shims equally spaced from $G_y = -0.8$ to $G_y = 0.8$ mT m⁻¹ and nine Y-shims from $G_x = -1.2$ to $G_x = 1.2$ mT m⁻¹ were used. The Z-shim was incorporated into the slice-rephase gradient which lasted 2 ms and the Y-shim was played at the same time. The ASE scan lasted for 16 minutes and 12 seconds.

**Image processing and analysis**

Image processing was carried out using a combination of FSL 5.0.1 \cite{29}, ANTs 2.1.0 \cite{10} and QUIT 3.1 \cite{13}. Briefly, the complex MP2-RAGE structural images were first coil-combined \cite{35} and then converted into both a T1 map and a uniform contrast image \cite{32}. From these, a study-specific template image was constructed \cite{13} which in turn registered to an atlas image \cite{34}. Eleven bilateral Regions Of Interest (ROIs) were selected from the atlas and transformed to the template space; the Thalamus (Thl), Hypothalamus (HThl), Striatum (Stri), Inferior Colliculus (Infc), Cingulate Cortex (CgCx), Retrosplenial Cortex (RtCx), Insular Cortex (InCx), Corpus Callosum (Cc), Septum (Sptm), Dorsal Hippocampus (Dhip) and Peri-Aqueductal Grey Matter (PAG).

The CASL images were corrected for motion \cite{35} and susceptibility distortions \cite{40}, and then converted into a CBF map using the BASIL tool \cite{37}. The T1 of blood was set to 2.429 s \cite{40}, the labelling efficiency was set to 80%, and the distortion-corrected reference image was used as the proton density during CBF quantification \cite{35}. The reference image was registered to the MP2-RAGE structural image.

The ASE images with different Z- & Y-shims were first combined by taking the Root Sum-of-Squares (RSS). To avoid noise amplification artefacts, we calculated the mean squared noise amplification artefacts, we calculated the mean squared intensity in a background region and subtracted this from sum-of-squared images before taking the square root of the difference. The resulting intensity in the readout (X, left-right) direction, we introduced

\[ R_{c} \delta_s \Delta T, \] from which the parameters $T_{c}$, dHb and most importantly OEF could be derived. The resulting OEF and CBF maps were then multiplied together and by $C_{r}$ to produce the CMRO\textsubscript{2} map. The parameter maps were resampled into the template space and average ROI values extracted using the template-specific masks.

**Autoradiography protocol and analysis**

To assess regional brain glucose metabolism we performed \textsuperscript{1}C-2-deoxyglucose (2DG) autoradiography, which measures Glucose Utilisation (GU) in µM/100g/min as originally described.
by Sokoloff[41]. We used a separate cohort of ten adult male Sprague Dawley rats (weight 325–380 g). All were initially anaesthetised for approximately 30 minutes with 2.5–3% isoflurane (in 80/20 medical air/oxygen), in order to cannulate their femoral and tail blood vessels for blood sampling and compound administration, respectively. After the cannulation, a local anaesthetic was applied and the wound sutured.

Isoflurane was then set to 2.5% for five rats. In the remaining rats, isoflurane was terminated and an intravenous bolus of 65 mg kg\(^{-1}\) α-Chloralose was administered, followed by 30 mg kg\(^{-1}\) h\(^{-1}\) infusion for the remainder of the experiment\(^{42}\). Body temperature was maintained at 36 ± 0.5°C using a thermostatically controlled electric heating blanket and rectal probe.

Between 30 and 40 minutes was allowed for the rats to stabilise, after which we intravenously administered over 30 s 100 µCi/kg 2DG (Perkin Elmer, USA), and collected 14 timed arterial blood samples\(^{43}\) over 45 minutes. After the final blood sample the animals were decapitated. Their brains were removed and frozen in −40 °C isopentane and then stored at −80 °C. Quantification of plasma glucose and \(^{14}\)C was carried out using a blood glucose analyser (YSI 2300) and scintillation counter (Beckman Coulter LS 6500), respectively. Brains were cryosectioned at 20 µm and exposed to X-ray film (Kodak Biomax MR-2) alongside calibrated \(^{14}\)C standards (GE Healthcare UK) for 7 days, after which they were developed in an automated X-ray film processor. Images were digitised using a Nikon single lens reflex camera and a macro lens, over a Northern Lights illuminator (InterFocus Ltd UK). Brain GU was calculated from the optical densities in the films using a calibration curve and the plasma glucose levels according to 41. We measured GU in eleven ROIs which matched those chosen from the MRI atlas, located at approximately +1, -3.5 and -8 mm from Bregma\(^{44}\). Readings for each ROI were taken bilaterally from two or three adjacent brain sections and then averaged. The analyst was blinded to anaesthetic group.

Statistical analysis

For statistical analyses we used the Python libraries pandas 1.0.5 and statsmodels 0.11.1\(^{45}\). The mean ROI values for each anaesthetic were compared with a non-parametric Mann-Whitney U-test with False Discovery Rate (FDR) multiple-comparisons correction. Finally, we compared our MRI oxygen metabolism measurements to the glucose metabolism measurements using a Robust Linear Model analysis of CMRO\(_2\) against GU. In this model, the slope of the line is the number of oxygen molecules consumed per molecule of glucose during metabolic activity, while the intercept gives the amount of oxygen consumed if no glucose was being consumed. Robust regression was used because residual variance was inhomogenous across the metabolic range. As our experimental design did not use the same animals for both CMRO\(_2\) and GU experiments, the measurements for each ROI were averaged across subjects (but not anaesthetics) before the regression, yielding a total of 22 data points for this analysis. For all analyses, a p-value of less than 0.05 was considered significant. ROI data and group average data are available in Underlying data\(^{46}\).
extreme shims are mostly noise, whereas in Figure 2 the unshimmed image is mostly noise and the signal has shifted towards negative values of $G_y$ and $G_Z$.

Figure 3 shows the result of combining all the different shim images via RSS both with and without noise suppression. Without suppression, amplification of the Rician noise is so severe that the background has almost the same intensity as the image. Subtracting the mean squared background intensity before the square-root operation restores the correct noise properties to the image, with crisp contrast between the image and background regions.

**Group comparisons**

Figure 4 shows the results of the model fit to the shimmed ASE data. $R'_2$ appears slightly higher in animals anaesthetised with $\alpha$-Chloralose. Residual elevated $R'_2$ can be observed surrounding the mastoid cavities and in a thin layer around the brain, where the $Z$-&$Y$-shimming was insufficient to correct extreme MFGs. The Root Mean Square Error (RMSE) is flat across most of the brain, indicating a reasonable model fit, but is elevated in white matter and cerebrospinal fluid (CSF), indicating the model fits less well in these areas. $\Delta T$ is increased towards the lower front of the brain.

Figure 5 shows the mean OEF, CBF and CMRO$_2$ for isoflurane and $\alpha$-Chloralose anaesthesia. The OEF is higher under $\alpha$-Chloralose. Areas with elevated $R'_2$ due to MFGs also show artefactually high OEF. CBF is much lower under $\alpha$-Chloralose anaesthetic than under isoflurane. The Inferior Colliculus shows an elevated CBF compared to other brain regions. CMRO$_2$ is consistently higher under isoflurane than under $\alpha$-Chloralose.

In Figure 6 we display glucose consumption under both anaesthetics. Similarly to the MRI data, glucose metabolism is clearly reduced under $\alpha$-Chloralose compared to isoflurane, and the Inferior Colliculus displays elevated metabolism compared to the rest of the brain.

Table 1 gives the mean and standard deviation across subjects of each ROI for OEF, CBF, CMRO$_2$, and GU. Figure 7 shows the same data plotted graphically. CMRO$_2$, GU and CBF were all lower under $\alpha$-Chloralose than isoflurane, while OEF was higher under $\alpha$-Chloralose than isoflurane. These effects were strong and consistent, with perfect separation between $\alpha$-Chloralose and isoflurane (i.e. all values in one group higher/lower than the other) apart from the following exceptions: for OEF in the Striatum and Hypothalamus (FDR-corrected $p = 0.1$ & 0.14 respectively), for CMRO$_2$ in the Cingulate Cortex (FDR-corrected $p = 0.21$) and for GU in the Inferior Colliculus (FDR-corrected $p = 0.09$).

Finally we show the result of regressing CMRO$_2$ against GU for the different regions of interest (averaged across subjects) in Figure 8. The slope of the line of best fit was 6.4 ($p < 0.001$, 95% CI 4.80 to 8.00), while the fitted intercept of $-77.3 \, \mu$M/100g/min was not significantly different from zero ($p = 0.174$, 95% CI $-188.75$ to $34.22$).

**Discussion**

The above results demonstrate that CMRO$_2$ can be measured in rats using a combination of ASE and ASL images. The method does not require administration of a gas challenge or administration of expensive isotopes. Hence this method has the potential to be a cheap, easily available method compared to gold-standard PET measurements.
There were numerous technical challenges to implementing the ASE method at ultra-high field (9.4T) and the small dimensions of a preclinical system compared to previous clinical work. Foremost, MFGs were highly problematic, and adequately correcting them involved a large number of trade-offs which prevented full correction across all regions of the brain. Notably, we observed strong gradients in all three geometric directions. This required the implementation of shimming in both the slice-select (Z) and phase-encode (Y) directions. Providing an adequate number of shims required acquiring a total of 45 images per value of $\tau$ (nine Y-shims multiplied by 5 Z-shims), which is significantly more than the eight images that were adequate in a clinical setting\textsuperscript{19}. Including shim gradients in the readout direction (X) may have further reduced MFG artefacts, but at the expense of additional scan-time. Thinner slices would also reduce the impact of the MFGs, but would also lower signal-to-noise ratio (SNR) and brain coverage. Acquiring more slices would be problematic for the ASL scan, where the maximum number is determined by the time between the end of the post-labelling time and the end of TR. Increasing TR and hence...
Figure 5. Slices through the mean Oxygen Extraction Fraction (OEF), Cerebral Blood Flow (CBF) and Cerebral Metabolic Rate of Oxygen (CMRO₂) for both anaesthetics. CMRO₂ is lower under α-Chloralose, however this is driven by a significant reduction in CBF as OEF is actually higher under α-Chloralose than isoflurane. Note that the slice through the inferior colliculus for CMRO₂ has a different color scale due to the much higher rate of metabolism compared to the other slices.

Figure 6. Glucose consumption measured with autoradiography under (left) isoflurane and (right) α-Chloralose. GU: Glucose Utilisation.

the number of slices would hence increase the ASL scan time further and lead to very different post-labelling times for different slices.

As shown in Figure 2, naïve RSS combination of the different shims leads to amplification of the Rician noise in low signal areas. We could not use the Fourier Transform approach to shim combination taken by Stone & Blockley as the necessary reconstruction methods were not available from the manufacturer. Subtracting the average noise level from the squared magnitude images restored an adequate level of SNR. Despite this, we found we could not reliably fit a value of DBV to our data. This is likely because the highest value of τ we could achieve, 56 ms, was not high enough to provide a stable fit to both $R_2'$ and DBV simultaneously. Increasing the maximum value of τ would necessitate either a corresponding increase in TE, which would reduce SNR and increase the effects of MFGs, or the use of Partial Fourier acceleration, which we found caused unacceptable blurring and intensity artefacts from the echo moving out of the acquisition window. The introduction of the parameter ΔT, representing either the early or late arrival of the spin-echo peak, improved the stability of our fit on the edges of white matter and towards the lower front portion of the brain. It is not clear what physical process may be causing this.

OEF can also be measured with MRI using the quantitative Blood Oxygenation Level Dependent (qBOLD) method. This has been previously applied in rodents at 4.7 Tesla, but required a scan-time of one hour. In humans, qBOLD has been combined with QSM to estimate CMRO₂ from a single multi-echo gradient-echo scan. This method shows promise but the required modelling and processing was extremely complex. In contrast, after correction for MFGs, the ASE method...
Table 1. Mean and standard deviation of each parameter value in each Regions of Interest (ROI), and the average across the ROIs. OEF, Oxygen Extraction Fraction; CMRO₂, Cerebral Metabolic Rate of Oxygen; CBF, Cerebral Blood Flow; GU, Glucose Utilisation.

<table>
<thead>
<tr>
<th>ROI</th>
<th>OEF (%)</th>
<th>CBF (ml/100g/min)</th>
<th>CMRO₂ (µM/100g/min)</th>
<th>GU (µM/100g/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Iso</td>
<td>αCl</td>
<td>Iso</td>
<td>αCl</td>
</tr>
<tr>
<td>Stri</td>
<td>14.4 ± 0.5</td>
<td>17.8 ± 1.9</td>
<td>139.8 ± 12.2</td>
<td>61.5 ± 2.7</td>
</tr>
<tr>
<td>CnCx</td>
<td>11.5 ± 1.1</td>
<td>20.0 ± 3.2</td>
<td>137.5 ± 10.8</td>
<td>44.1 ± 3.0</td>
</tr>
<tr>
<td>CC</td>
<td>17.2 ± 0.6</td>
<td>21.3 ± 1.1</td>
<td>92.4 ± 8.9</td>
<td>39.1 ± 0.5</td>
</tr>
<tr>
<td>RtCx</td>
<td>11.4 ± 1.4</td>
<td>18.9 ± 0.7</td>
<td>144.3 ± 10.2</td>
<td>55.4 ± 2.9</td>
</tr>
<tr>
<td>Thl</td>
<td>17.5 ± 1.5</td>
<td>22.8 ± 1.3</td>
<td>150.8 ± 19.3</td>
<td>49.0 ± 1.6</td>
</tr>
<tr>
<td>InCl</td>
<td>25.9 ± 1.1</td>
<td>31.6 ± 1.9</td>
<td>209.6 ± 19.7</td>
<td>69.7 ± 6.0</td>
</tr>
<tr>
<td>InCx</td>
<td>17.8 ± 1.0</td>
<td>25.5 ± 2.9</td>
<td>140.3 ± 11.3</td>
<td>54.8 ± 1.8</td>
</tr>
<tr>
<td>Sptm</td>
<td>10.9 ± 0.4</td>
<td>14.8 ± 0.5</td>
<td>124.7 ± 14.0</td>
<td>45.6 ± 1.1</td>
</tr>
<tr>
<td>HThl</td>
<td>18.0 ± 1.3</td>
<td>16.8 ± 0.6</td>
<td>149.0 ± 13.0</td>
<td>51.6 ± 1.8</td>
</tr>
<tr>
<td>DHIp</td>
<td>13.0 ± 1.2</td>
<td>23.9 ± 3.3</td>
<td>129.5 ± 10.2</td>
<td>48.2 ± 1.5</td>
</tr>
<tr>
<td>PAG</td>
<td>14.8 ± 0.9</td>
<td>18.5 ± 0.9</td>
<td>171.5 ± 17.3</td>
<td>61.9 ± 4.5</td>
</tr>
<tr>
<td>Avg</td>
<td>15.7 ± 0.7</td>
<td>21.1 ± 0.8</td>
<td>144.5 ± 13.2</td>
<td>52.8 ± 1.5</td>
</tr>
</tbody>
</table>

Figure 7. Mean value of Oxygen Extraction Fraction (OEF), Cerebral Blood Flow (CBF), Cerebral Metabolic Rate of Oxygen (CMRO₂), and Glucose Utilisation (GU) in the chosen Regions of Interest (ROIs) for each subject. CMRO₂ and GU consumption are both reduced under α-Chloralose anaesthetic compared to isoflurane. Almost total separation between the two groups was achieved; ROIs and parameters where this did not occur are noted in the text.
We used the averaged regions of interest data for both anaesthetics. Accordingly, our CMRO$_2$ measurements confirmed the expected differential effect of the anaesthetics on cerebral metabolism, with close to double the rate of oxygen consumption under isoflurane than $\alpha$-Chloralose. We note that the difference in CMRO$_2$ was driven primarily by the difference in CBF which was three times higher under isoflurane, while OEF only reduced by a quarter compared to $\alpha$-Chloralose. This is in line with the notion that mitochondria require a particular gradient of tissue oxygenation, and because less oxygen is removed from the blood during higher flow (decreased capillary transit time), it follows that OEF is decreased with increased CBF and CMRO$_2$.

Finally we compared our MRI measure of CMRO$_2$ to a gold-standard measure of glucose metabolism by autoradiography. As expected, glucose consumption was significantly different between the two anaesthetics. By averaging within ROI across subjects we were able to perform a regression analysis of CMRO$_2$ against GU. The resulting slope of this line is expected to be six, corresponding to the stoichiometric ratio of six molecules of oxygen consumed for every molecule of glucose during aerobic metabolism. Our value of 6.4 is very close to this, and the expected value is within the confidence interval of our measurement, suggesting that both our MRI and autoradiography measurements are reasonably accurate.

Conclusions

We implemented a non-invasive method of measuring CMRO$_2$ using MRI in rats which can be easily translated to clinical scanners. Although methodological difficulties prevented measurement of DBV, we successfully demonstrated the method by comparing brain metabolism under two common anaesthetics. Our values of OEF, CBF, and CMRO$_2$ are comparable to literature values and were in alignment with glucose consumption measured with autoradiography.

Data availability

Underlying data


This project contains the following underlying data:
- ROIs (ROI summary statistics in Comma Separated Value format).
- Parameter maps (mean parameter maps in Comma Separated Value format).

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

References


Figure 8. A regression analysis of Cerebral Metabolic Rate of Oxygen (CMRO$_2$) against Glucose Utilisation (GU) across the averaged regions of interest data for both anaesthetics.
http://www.doi.org/10.6084/m9.figshare.14199035

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Yolanda Ohene
The University of Manchester, Manchester, UK

Wood et al. have combined two techniques, arterial spin labelling (ASL) and asymmetric spin-echo (ASE), that capture the cerebral blood flow (CBF) and the oxygen extraction fraction (OEF), respectively, to provide a non-invasive measurement of cerebral metabolic rate of oxygen (CMRO$_2$).

CMRO$_2$ measurements were taken in rodents under two different anaesthetic conditions. The authors demonstrate that CMRO$_2$ is lower under α-Chloralose anaesthesia compared to isoflurane anaesthesia. The metabolic activity was validated by measuring glucose consumption using autoradiography under the same two anaesthetic conditions. The glucose metabolic rate is also lower under α-Chloralose anaesthesia. The study design resulted in a clear separation of the OEF, CBF, CMRO$_2$ and glucose utilisation (GU) under the two anaesthetic conditions, in almost all brain regions, demonstrating the successful implementation of this non-invasive method.

Minor comments:
- Could the authors comment on the effect of fixing the deoxygenated blood volume (DBV) in the OEF measurements, particularly considering that there is a significant difference in the CBF between the two anaesthesia groups which may have an influence on the DBV value?
- Figure 5: Labelling of the coloured bars are overlapping on the bottom row images.

Is the rationale for developing the new method (or application) clearly explained?
Yes

Is the description of the method technically sound?
Yes
Wood et al. have presented a new \( R_2' \)-based method for quantifying the oxygen extraction fraction (OEF) and the cerebral metabolic rate of \( \text{O}_2 \) (CMRO\(_2\)) in rats. The novelty here lies in i) the variable shimming approach used to reduce the effect of macroscopic field inhomogeneities that otherwise contaminate the contribution to \( R_2' \) from deoxyhemoglobin in the vasculature, ii) the application of this approach to compare various brain physiological parameters (OEF, CBF, CMRO\(_2\)) under two different anesthetics (isoflurane and alpha-Chloralose) and iii) the comparison of CMRO\(_2\) to the glucose uptake (GU) by autoradiography.

The study shows large differences in brain physiology under the different anesthetics, consistent with previous findings. The final finding that the authors fit a CMRO\(_2\) to GU ratio of 6.4 is quite remarkable, considering the theoretical ratio is 6 (i.e., 6 molecules of \( \text{O}_2 \) consumed per glucose during oxidative glycolysis). I do have significant concerns, however, with the quantification of \( R_2' \) given that multiple previously unpublished techniques were employed that I think would benefit from further validation. Given that the OEF and CMRO\(_2\) quantification rely on the estimate of \( R_2' \), this does have important consequences for the comparisons of the absolute OEF and CMRO\(_2\).
values across the two anesthetics and for the absolute CMRO$_2$::GU ratio.

1. To the best of my knowledge, the way the shimmed images were combined through root sum of squares is a previously unpublished approach. Similarly, the proposed noise floor correction via subtraction of the mean-squared noise signal in the background is also novel to this study. While the noise subtraction does appear to improve the contrast-to-noise, it is not apparent that the desired absolute signal levels are preserved. Since these images are later used for absolute quantification of $R_2^*$, it is important that the signal levels are not systematically biased, but if they are, at least that bias can be characterized. It would be reassuring to see a validation of these new techniques.

2. The authors introduced the $R_2^*$ fitting parameter $\Delta T$ to account for shifts in the signal maximum away from the nominal spin-echo time at $\tau = 0$. The magnitude of the shifts, ±25 ms, are dramatic and are previously unheard of (to the best of my knowledge). This is possibly related to the shimming and/or the shim image combination. Again, some form of external validation of this parameter to better understand its origin would help give better trust in the resulting $R_2^*$ fits.

3. Another major limitation of this study was that the $R_2^*$ fitting required fixing the value of deoxygenated blood volume (DBV) to 3.3%. While this may have helped stabilize the fit, it would lead to OEF estimates that would differ from their true values depending on how the true DBV compared to the assumed value, and this points to issues with the model and/or data. As DBV is regionally varying, this limits the validity of the OEF estimates. Could the authors elaborate on how they performed the fitting? In Eq. 2, there is the quadratic decay period for $\tau < T_c$ and the linear decay period for $\tau > T_c$. Also note, typically studies have used a factor of 1.5*$T_c$ for the transition period, although this would depend on how the authors have defined $T_c$ (e.g., refs. 17 or 19). Was data from all periods fit? How was $T_c$ determined without a priori knowing $R_2^*$? In previous quantitative BOLD studies, the linear decay period is extrapolated to the signal at $\tau = 0$ and the difference between this signal and the measured signal is proportional to DBV, was this fitting approach used here?

Minor comments:

1. In the opening paragraph, the authors refer to “quantitative BOLD” using calibration with gases. This calibration with gases is referred to as “calibrated” BOLD or fMRI, not quantitative BOLD. Quantitative BOLD is a gas-free technique which the methods in this manuscript are based on.

2. The values for the arterial O$_2$ concentration, Ca, are based on human physiological parameters. The authors should consider using values for rats. See, e.g., Gagnon et al., J Neurosci (2015).$

3. The labels are cropped in Figure 5

4. In the middle of the Discussion section, the authors compare their OEF estimates to He et al.’s where they state findings that were in line with the findings of this manuscript. I think that stating the results being in line with each other is a bit generous as the OEF values in the manuscript differ by 35% (iso.) and 44% (alpha-chlor.) relative to those from He et al.

5. It would be nice to see a discussion of other contributions to $R_2^*$ contamination beyond
macroscopic field inhomogeneities, such as iron depositions or regionally varying myelination since these may also bias the OEF estimate and would be important to be aware of in (pre)clinical studies.

6. It would be helpful to label some of the key ROIs discussed in the manuscript, in particular the inferior colliculus.

References

Is the rationale for developing the new method (or application) clearly explained?
Yes

Is the description of the method technically sound?
Partly

Are sufficient details provided to allow replication of the method development and its use by others?
Partly

If any results are presented, are all the source data underlying the results available to ensure full reproducibility?
Yes

Are the conclusions about the method and its performance adequately supported by the findings presented in the article?
Partly

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** biophysical modelling of the blood oxygenation level-dependent (BOLD) fMRI signal, high-resolution fMRI, MRI pulse sequence development

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

**Author Response 09 Sep 2021**

**Tobias C. Wood,** Institute of Psychiatry, Psychology & Neuroscience, King’s College London, UK

We thank the reviewer for their constructive feedback. Responses to the individual points are below.
Major:

1. A key reference describing the use of the root-sum-squares method for combining z-shimmed images (Spencer & Constable 1999) was omitted. This has now been corrected. Similarly, the noise floor correction method has also been previously published (Miller & Joseph 1993, reference 40) where it was used in the context of T2/R2 fitting. While these techniques are old to our knowledge they have not been invalidated.

2. We agree with the reviewer that such shifts have not been described previously in the literature, however the linear extrapolation method used by Blockley et al cannot detect such shifts even if they are present. In addition, previous literature in this area has used human subjects and lower field strengths. We suspect that strong MFGs in the x-direction, which we could not compensate due to scan time constraints, may be the underlying cause of such shifts. This putative explanation has been added to the discussion, along with an additional reference (Chen et al)

3. We agree that fixing the value of DBV is a limitation of this study. We previously used a value of 1.5Tc for the transition period and Tc=R2/DBV and a non-linear fit across all data points. Whave now updated the code (and relevant equations in the manuscript) to use the full integral form of Yablonskiy et al, but note that this made very little difference to the fitted values compared to the asymptotic form of the equations. This code is now available in version 3.3 of our toolbox. Likely, a major contributor to the difficulty in fitting for DBV is the relatively small maximum value of we could achieve within imaging constraints and only having a single t=0 image. A more efficient protocol may be to acquire multiple t=0 images and fewer intermediate values of t. This point has been expanded in the discussion.

Minor comments:

1. Thank you for the clarification, the introduction has been reworded accordingly.

2. We thank the reviewer for bringing this to our attention. Please see the notes on the amendments at the start of the text for a full discussion of this issue.

3. The labels have been corrected in figure 5.

4. On reflection we agree with the reviewer. The sentence has been amended to state that the values in He et al are further evidence that our method currently underestimates OEF.

5. Discussion of the effect of myelination has been added, as it is clear the current model does not account for this.

6. Due to the 3D nature of the ROIs, displaying all of them would require an additional figure. The Inferior Colliculus has been marked on an existing figure with arrows.

Competing Interests: No competing interests were disclosed.