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

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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\(^1\). There is great interest in being able to quantitatively map the Cerebral Metabolic Rate of Oxygen (CMRO\(_2\)) consumption, both as a marker of pathology and for the study of healthy ageing\(^2,3\). Although methods exist using oxygen isotopes with either Magnetic Resonance (MR) spectroscopic imaging or Positron Emission Tomography (PET)\(^4,5\), 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 CMRO\(_2\) using a combination of T2-mapping and phase-contrast velocity measurements\(^6,7\), voxel-wise mapping using quantitative Bloody Oxygenation Level Dependent (qBOLD) with calibration from gas administration\(^8,9\) and high-resolution mapping methods based on Quantitative Susceptibility Mapping (QSM)\(^10,11\).

For this study we created a straightforward and robust method to measure CMRO\(_2\) 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)\(^12\). OEF maps were constructed by measuring the reversible rate of transverse relaxation R\(_2^\prime\), which is related to the concentration of deoxyhaemoglobin (dHb)\(^13-15\).

We demonstrated our method by imaging rats with two anaesthetics known to affect brain metabolism differently, and validated these MRI measurements by comparing them to gold-standard autoradiography measurements of glucose metabolism under the same anaesthetics.

Methods
Ethics statement
Study procedures were conducted in accordance with the Animal (Scientific Procedures) Act 1986 and with ethical approval from the King’s College London Animal Welfare And Ethical Review Body (AWERB) under the authorisation of license number P023CC39A. All harm to animals was prevented as procedures were performed under anaesthesia. Animals were group housed under standard laboratory conditions with freely available food and water. There were no exclusion criteria for the animals.

Theory
CMRO\(_2\), here measured in µM/100g/min, is defined as the product of CBF, measured in ml/100g/min, and OEF multiplied by the constant C\(_a\):
\[
\text{CMRO}_2 = \text{CBF} \times \text{OEF} \times C_a
\]

C\(_a\) describes the the amount of oxygen carried by arterial blood and is measured in µM ml\(^{-1}\). It can be calculated as C\(_a\) = φ × SaO\(_2\) × [Hb], with the carrying capacity of haemoglobin φ = 1.34 mlO\(_2\)g\(_Hb\)^{-1}, the arterial oxygen saturation SaO\(_2\) = 98% and the haemoglobin concentration [Hb] = 15gmL\(^{-1}\).\(^12\).

The measurement of CBF (measured in ml/100g/min) with ASL is a well-established MR method\(^6,12,20\). We chose to measure OEF from R\(_2^\prime\), which is defined as the difference between the combined relaxation rate R\(_2\) and the irreversible relaxation rate R\(_2^\prime\) (R\(_2^\prime\) = R\(_1\) + R\(_2\)), where relaxation rates are the inverses of relaxation times (R\(_2\) = 1/T\(_2\)). MRI images can be acquired with T\(_2^\prime\)-weighting using an Asymmetric Spin-Echo (ASE) sequence where the refocusing pulse is offset from the standard time to produce a spin echo, T\(_2/2\), by an echo-shift τ/2, which can be either positive (the pulse occurs later than T\(_2/2\)) or negative (the pulse occurs earlier than T\(_2/2\)). Echoes formed at the same T\(_2\) but different τ will hence have the same T\(_2\)-weighting, but different amounts of additional T\(_2^\prime\) or R\(_2^\prime\) weighting. By observing the signal in each voxel from multiple τ values, we can measure a mono-exponential R\(_2^\prime\) as we would measure R\(_1\) from multiple values of T\(_2^\prime\).

However, in brain tissue the observed signal value at τ = 0 is less than would be expected from extrapolating the signal curve for τ ≠ 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\(^16\). In this model, the dephasing only affects the signal evolution below the critical time τ = T\(_c\). The signal S can hence be described in two parts:
\[
S(\tau) = \begin{cases} 
S_0 \exp \left( -0.3(R_2^\prime \tau)^2 \right) & \tau < T_c \\
S_0 \exp \left( -R_2^\prime \tau + \text{DBV} \right) & \tau > T_c 
\end{cases}
\]

(2)

These equations are equivalent to those used by Stone & Blockley\(^17\), but formulated purely in terms of R\(_2^\prime\) and Deoxygenated Blood Volume (DBV) and we have neglected the dependence of S\(_0\) on TE and T\(_c\) for clarity. The OEF can then be found by
\[
\text{OEF} = \frac{3R_2^\prime}{4\pi \text{DBV} \gamma B_0 \delta \chi_0 \text{Hct}}
\]

(3)

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]\(^18\). In previous clinical studies it has been possible to estimate DBV from the ASE data\(^19\). 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\(^11\).

R\(_2^\prime\) 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{19,20}. 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{21,22}. 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{23}. 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 by inhaling 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{24}, 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 \(\alpha_1/\alpha_2 = 7/9^\circ\). An additional Utrashort Echo Time (UTE) COMPOSER scan was acquired for coil combination\cite{25}.

For ASL we used the manufacturer’s Continuous ASL (CASL) sequence with a spin-echo Echo Planar Imaging (EPI) readout\cite{26}. 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{27,28}, 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{29}. Twelve values of \(\tau\) spaced from -32 to 56 ms were acquired. At each, five Z-shims equally spaced from \(G_z = -0.8\) to \(G_z = 0.8\) mT m\(^{-1}\) and nine \(\gamma\)-shims from \(G_y = -1.2\) to \(G_y = 1.2\) 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{30}, ANTs 2.1.0\cite{31} and QUIT 3.1\cite{32}. Briefly, the complex MP2-RAGE structural images were first coil-combined\cite{33} and then converted into both a T1 map and a uniform contrast image\cite{34}. From these, a study-specific template image was constructed\cite{35} which in turn registered to an atlas image\cite{36}. 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{37} and susceptibility distortions\cite{38}, and then converted into a CBF map using the BASIL tool\cite{39}. 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{41}. 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 intensity in a background region and subtracted this from sum-of-squared images before taking the square root\cite{42}. To avoid noisy to reliably fit for the parameter DBV, which is thought to be on the order of a few percent. To improve the quality of the fit for the remaining parameters we hence fixed DBV = 3.3%\cite{43}. We also observed that in certain brain regions the peak of our signal curve did not occur precisely at \(\tau = 0\), hence we introduced an additional parameter \(\Delta T\) to account for this. The final free parameters were \(K'_{c}\), \(S_c\) and \(\Delta T\), from which the parameters \(T_s\), \(dHb\) and most importantly OEF could be derived. The resulting shimmmed ASE images were then motion and distortion corrected using the ASL reference data. The OEF was found from the corrected data by a non-linear fit to Equation 2 implemented in QUIT\cite{44}. We found that our images were too noisy to reliably fit for the parameter DBV, which is thought to be on the order of a few percent. To improve the quality of the fit for the remaining parameters we hence fixed DBV = 3.3%\cite{45}. We also observed that in certain brain regions the peak of our signal curve did not occur precisely at \(\tau = 0\), hence we introduced an additional parameter \(\Delta T\) to account for this. The final free parameters were \(K'_{c}\), \(S_c\) and \(\Delta T\), from which the parameters \(T_s\), \(dHb\) and most importantly OEF could be derived. The resulting OEF and CBF maps were then multiplied together and by \(C_c\) to produce the CMRO\(_2\) 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 \(^{1}C\)-2-deoxyglucose (2DG) autoradiography, which measures Glucose Utilisation (GU) in \(\mu M/100 g/min\) as originally described.
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. 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 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. 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. 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.

Results
Pre-processing
Figure 1 and Figure 2 show a single slice through all the raw ASE images collected with different values of Z- & Y-shims at $\tau = 0$ and $\tau = 56$ ms, respectively. The central images have both $G_y$ and $G_z$ equal to zero, i.e. in Figure 1 this is a simple unshimmmed symmetric spin-echo image. In Figure 1 only the central, low value shims contain significant signal and the

Figure 1. Raw asymmetric spin-echo data in a single slice at $\tau = 0$ ms for all the values of Z- & Y-shims. The signal is concentrated at low shim values as expected.
extreme shims are mostly noise, whereas in Figure 2 the unshimmed image is mostly noise and the signal has shifted towards negative values of \( G_x \) and \( G_y \).

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 CMRO₂ for isoflurane and \( \alpha \)-Chloralose anaesthetic. 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. CMRO₂ 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, CMRO₂ and GU. Figure 7 shows the same data plotted graphically. CMRO₂, 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 CMRO₂ 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 CMRO₂ 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 CMRO₂ can be measured in rats using a combination of ASE and ASL images. The method does not require administration of a gas challenge\(^{47,48} \), or administration of expensive isotopes\(^7 \). 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\(^1\). 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 (CMRO$_2$) for both anaesthetics. CMRO$_2$ is lower under $\alpha$-Chloralose, however this is driven by a significant reduction in CBF as OEF is actually higher under $\alpha$-Chloralose than isoflurane. Note that the slice through the inferior colliculus for CMRO$_2$ 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) $\alpha$-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$^{19}$ 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 $\tau$ 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 $\tau$ would necessitate either a corresponding increase in TE, which would reduce SNR and increase the effects of MFGs$^{19}$, 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$^{28}$. The introduction of the parameter $\Delta T$, 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$^{21}$. In humans, qBOLD has been combined with QSM to estimate CMRO$_2$ from a single multi-echo gradient-echo scan$^{15}$. 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; CMRO\textsubscript{2}, 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>CMRO\textsubscript{2} (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>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>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>
</tbody>
</table>

Figure 7. Mean value of Oxygen Extraction Fraction (OEF), Cerebral Blood Flow (CBF), Cerebral Metabolic Rate of Oxygen (CMRO\textsubscript{2}), and Glucose Utilisation (GU) in the chosen Regions of Interest (ROIs) for each subject. CMRO\textsubscript{2} 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.
only requires a fit of Equation (2) to the data. To estimate \( R' \), accurately we fixed the value of DBV. The chosen value of DBV acts as a scaling value that converts \( R' \) to OEF. We used the whole-brain value of 3.3% reported by He et al., who also reported mean OEFs of 23% and 38% under isoflurane and \( \alpha \)-Chloralose respectively, in line with our work.

We then combined our measurement of OEF with CBF measured by ASL to generate a map of CMRO\(_2\) under two common anaesthetics which are known to have different effects on brain metabolism. By using a dedicated labelling coil and correcting our multi-slice 2D data with the correct post-label delay we obtained full brain maps of CBF. Our values of CBF under isoflurane are similar to those reported previously using autoradiography. To scale CMRO\(_2\) correctly, we assumed a fixed value of SaO\(_2\) = 98% in line with previous clinical work. Our resulting values of CMRO\(_2\) under \( \alpha \)-Chloralose are in line with a previous study where they were measured using \( ^{17} \)O MR spectroscopic imaging.

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**


