

CHANGES IN THE ATTENUATION OF NEAR INFRARED SPECTRA BY THE HEALTHY ADULT BRAIN DURING HYPOXAEMIA CANNOT BE ACCOUNTED FOR SOLELY BY CHANGES IN THE CONCENTRATIONS OF OXY- AND DEOXY-HAEMOGLOBIN

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Abstract: It has been suggested that changes in oxidised cytochrome c oxidase concentration ([oxCCO]) measured using cerebral near infrared spectroscopy (NIRS) may be algorithm artefacts. We examine the change in near infrared (NIR) attenuation by the healthy adult brain (n=10) during hypoxaemia. Broadband spectroscopic data were collected during normoxia, and hypoxaemia. The UCL n algorithm was used to fit (a) oxy- (HbO₂) and deoxy-haemoglobin (HHb) spectra (2 component fit), and (b) HbO₂, HHb and oxidised-reduced cytochrome c oxidase difference spectra (3 component fit) to the mean change in NIR attenuation between baseline and hypoxaemia. The sum of squares of the residuals was 100×10^{-7} OD² for the 2 component fit and 8×10^{-7} OD² for the 3 component fit, and the two sets of residuals differed from each other (p=0.0003). We compare experimental and simulated data and suggest that the 2 component residuals indicate a change in [oxCCO]. Changes in near infrared attenuation by the healthy adult brain during hypoxaemia cannot be accounted for solely by changes in oxy- and deoxy-haemoglobin concentrations. Including [oxCCO] in the algorithm improves its fit quality. These data suggest that changes in cerebral cytochrome c oxidase redox occur during hypoxaemia and that they can be detected using NIRS.

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1. INTRODUCTION

The use of near infrared spectroscopy (NIRS) to measure changes in the optical characteristics of living tissue was first described by Jöbsis in 1977.¹ NIRS makes use of the fact that biological tissue is relatively transparent to light between 700-900 nm, thus allowing interrogation of structures beneath the tissue surface.¹ Light passing through biological tissue is subject to multiple scattering interactions, and this complicates the interpretation of the intensity of detected light. However, if light lost due to scattering is assumed to remain constant, and the average pathlength of light through tissue is known, the modified Beer-Lambert law can be used to convert change in light attenuation to absolute change in chromophore concentrations.²

In both animals and humans, NIRS has been used to measure change in concentrations of oxy- ($\Delta[\text{HbO}_2]$), and deoxy-haemoglobin ($\Delta[\text{HHb}]$) and oxidised cytochrome c oxidase ($\Delta[\text{oxCCO}]$),³⁻⁵ however controversy still remains as to the validity of the measured $\Delta[\text{oxCCO}]$.⁵

Cytochrome c oxidase (CCO) is the terminal electron acceptor of the mitochondrial electron transfer chain and catalyses over 95% of oxygen metabolism. The reduction of dioxygen provides the proton motive force to drive aerobic adenosine triphosphate synthesis.⁶ The difference spectrum between the oxidised and reduced forms of CCO has a distinct band in the near infrared (NIR) region, with a broad peak located around 830 nm.¹ Assuming the total concentration of CCO remains constant, changes in the CCO signal represent changes in the CCO redox state. The CCO signal is an attractive target for clinical monitoring, as it offers the potential to provide a non-invasive marker of the adequacy of mitochondrial oxygen delivery.

However, detection of changes in the CCO signal is complicated by the fact that the concentration of CCO in the brain is approximately one order of magnitude less than that of either oxy- or deoxy-haemoglobin.⁷ This raises the possibility that measured $\Delta[\text{oxCCO}]$ might simply be an artefact produced by the algorithms, used to convert measured attenuation changes into chromophore concentration changes, being unable to adequately separate the CCO and haemoglobin signals.⁵ Furthermore, controversy remains as to the degree of hypoxaemia required to produce changes in CCO redox state.

Despite these issues, $\Delta[\text{oxCCO}]$ has been shown to correlate with nuclear magnetic resonance ³¹P spectroscopy measured reduction in phosphocreatine and nucleoside triphosphate in an animal model of cerebral ischaemia,⁸ and in cardiac surgery in humans it has been shown to predict adverse neurological outcome.⁴

In this study we measure the change in NIR light attenuation by the healthy human brain during hypoxaemia using a broadband NIR spectrometer. We analyse the residual errors produced by the fitting procedure for the conversion of light attenuation into chromophore concentrations in order to determine if the change in NIR attenuation can be accounted for solely by $\Delta[\text{HHb}]$ and $\Delta[\text{HbO}_2]$, or whether $\Delta[\text{oxCCO}]$ must also be considered.

2. MATERIALS AND METHODS

This study was approved by the Joint Research Ethics Committee of the National Hospital for Neurology and Neurosurgery and the Institute of Neurology. We studied 10 healthy subjects (7 male, 3 female, median age 32 years, range 30-39). Broadband spectrometer (BBS) optodes were placed 3.5 cm apart in a black plastic holder, and fixed to the right side of the forehead in the midpupillary line. Light from a stabilised tungsten halogen light source was passed through 610nm long-pass and heat absorbing filters, and transmitted to the head via a 3.3mm diameter glass optic fibre bundle. Light incident on the detector optode was focused via an identical fibre bundle onto the 400 μm entrance slit of a 0.27m spectrograph (270M, Instruments SA, France) with a 300g/mm grating. NIR spectra between 650 and 980 nm were collected at 1Hz on a cooled charge coupled device detector (Wright Instruments, UK) giving a spectral resolution of $\sim 5\text{nm}$. An oximeter probe (Novametrix Medical Systems Inc., USA) measured arterial oxygen saturation (SaO_2). A modified anesthetic machine delivered gas to the subject via a mouthpiece. Inspired oxygen concentration (FiO_2) was measured using an inline gas analyser (Hewlett Packard, UK). The study commenced with five minutes monitoring at normoxia. We then added nitrogen to the inspired gases to induce a gradual fall in SaO_2 to 80% and, immediately after this was achieved, the FiO_2 was returned to normoxia for five minutes. This cycle was repeated three times.

The points just prior to the start of each hypoxaemia (baseline), and at the end of each hypoxaemia (hypoxaemia), were identified manually using the SaO_2 data. At each of the two points the mean of the preceding ten seconds of data was taken. Data from the three experimental cycles were averaged to give mean values for SaO_2 and NIR light intensity at baseline and hypoxaemia for each subject. Optical pathlength was calculated using second differential analysis of the 740nm water feature.⁹ Change in NIR attenuation was then calculated from:

$$\Delta A = \log_{10}(I_{base} / I_{hypox}) \quad (1)$$

where ΔA =change in attenuation from baseline to hypoxaemia, I_{base} =light intensity at baseline and I_{hypox} =light intensity at hypoxaemia measured in units of optical density (OD). The UCLn algorithm,¹⁰ a multiple regression analysis utilising the Beer-Lambert law was then used to fit chromophore extinction coefficients, corrected for the wavelength dependence of the optical pathlength¹¹, to the group mean change in attenuation, using 120 wavelengths between 780 and 900 nm. Chromophore specific extinction coefficients were downloaded from the medical physics UCL website.¹² First, only oxy- (HbO_2) and deoxy-haemoglobin (HHb) spectra (2 component fit – Eq. 2) and then HbO_2 , HHb and the oxidised-reduced CCO difference spectra (oxCCO) (3 component fit – Eq. 3) were fitted to the group mean change in attenuation. After interpolation of the residuals to the spectral resolution of the BBS (5 nm), the sums of the squares of the residuals from these two analyses were calculated, and the distributions of the two sets of residuals were compared.

$$\begin{pmatrix} \Delta[\text{HbO}_2] \\ \Delta[\text{HHb}] \end{pmatrix} = \frac{1}{PL} \begin{pmatrix} \epsilon_{\text{HbO}_2}(\lambda_i) & \epsilon_{\text{HHb}}(\lambda_i) \\ \vdots & \vdots \\ \epsilon_{\text{HbO}_2}(\lambda_j) & \epsilon_{\text{HHb}}(\lambda_j) \end{pmatrix}^{-1} \begin{pmatrix} \Delta A(\lambda_i) \\ \vdots \\ \Delta A(\lambda_j) \end{pmatrix} \quad (2)$$

$$\begin{pmatrix} \Delta[HbO_2] \\ \Delta[HHb] \\ \Delta[oxCCO] \end{pmatrix} = \frac{1}{PL} \begin{pmatrix} \varepsilon_{HbO_2}(\lambda_i) & \varepsilon_{HHb}(\lambda_i) & \varepsilon_{oxCCO}(\lambda_i) \\ \vdots & \vdots & \vdots \\ \varepsilon_{HbO_2}(\lambda_j) & \varepsilon_{HHb}(\lambda_j) & \varepsilon_{oxCCO}(\lambda_j) \end{pmatrix}^{-1} \begin{pmatrix} \Delta A(\lambda_i) \\ \vdots \\ \Delta A(\lambda_j) \end{pmatrix} \quad (3)$$

where $\Delta[HHb]$, $\Delta[HbO_2]$ and $\Delta[oxCCO]$ are changes in the concentrations of oxy-, and deoxy-haemoglobin and oxidised cytochrome c oxidase in μM , PL =pathlength in cm, ε is the specific extinction coefficient of the subsequent chromophore in $\text{OD}/\mu\text{M}/\text{cm}$ and ΔA is the change in attenuation, at wavelengths λ_i to λ_j .

We then produced a simulated attenuation spectrum calculated using assumed $\Delta[HHb]$, $\Delta[HbO_2]$ and $\Delta[oxCCO]$ and their respective specific extinction coefficients, and ignoring change in attenuation due to other chromophores, using Eq. (4). We fitted a 2 component model to this spectrum and compared the resultant residuals with those from a 2 component fit to the group mean experimental spectrum. 2 component fits to the experimental and simulated data for each individual were then compared.

$$\Delta A(\lambda_j) = PL\{\Delta[HbO_2] \times \varepsilon_{HbO_2}(\lambda_j) + \Delta[HHb] \times \varepsilon_{HHb}(\lambda_j) + \Delta[oxCCO] \times \varepsilon_{oxCCO}(\lambda_j)\} \quad (4)$$

Statistical analysis was carried out using SAS software (v8.2, SAS Institute, USA) and p values <0.05 were considered significant. Group changes between baseline and hypoxaemia were compared using Wilcoxon signed rank test and the distributions of the residuals from the various fitting procedures were compared using a 2 sample Siegel-Tukey test¹³.

3. RESULTS

Results are presented as median (interquartile range). The median time of hypoxia required to reach an SaO_2 of 80% was 4.48 mins (3.92 to 5.04). During the study SaO_2 fell from a baseline value of 99.0% (98.2 to 99.2) to 82.4% (80.1 to 84.7) at the end of hypoxaemia ($p=0.002$) (Figure 1). Note that due to the ten second averaging window this median SaO_2 is higher than 80%. There was no change in optical pathlength between baseline and hypoxaemia ($p=0.23$). Group mean change in attenuation from baseline to hypoxaemia is shown in Figure 2.

The residuals from the 2 and 3 component fits to the group mean experimental spectrum differed from each other ($p=0.0003$) (Figure 3). The sum of the squares of the residuals was $100 \times 10^{-7} \text{OD}^2$ for the 2 component fit to the experimental spectrum and $8 \times 10^{-7} \text{OD}^2$ for the 3 component fit to the experimental spectrum. There was no difference between the residuals from the 2 component fits to the experimental and simulated spectra ($p=0.61$) (Figure 3). The 2 component fits to the experimental and simulated data for each subject are shown in Figure 4. In eight out of the ten subjects, there were no differences between the two sets of residuals ($p>0.05$).

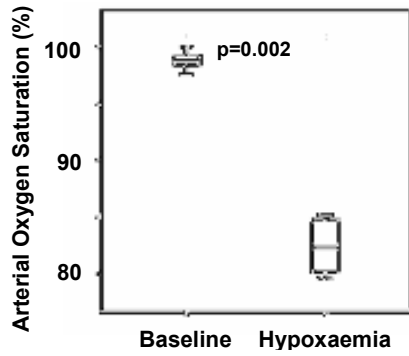


Figure 1. Boxplot showing arterial oxygen saturation data at baseline and hypoxaemia.

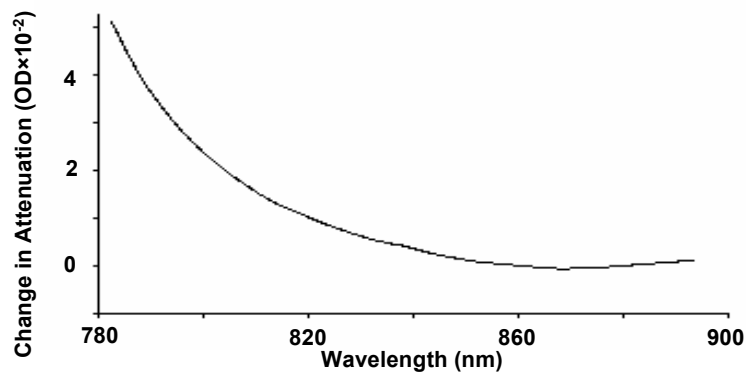


Figure 2. Group mean change in near infrared attenuation between baseline and hypoxaemia.

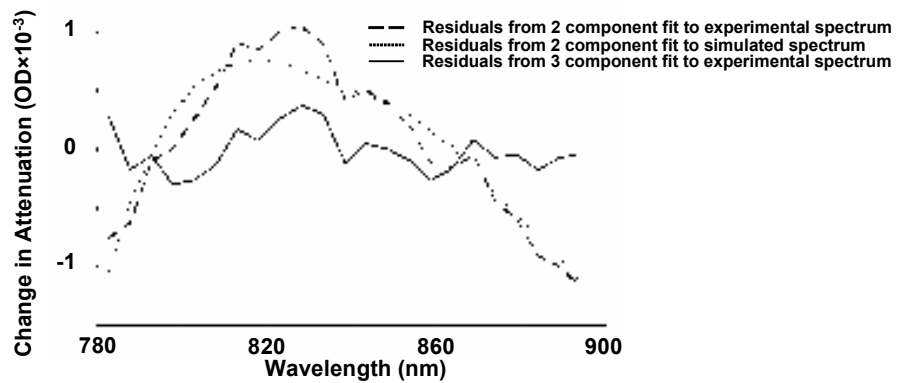


Figure 3. Residuals from 2 and 3 component fits to group mean change in near infrared attenuation between baseline and hypoxaemia and 2 component fit to simulated spectrum.

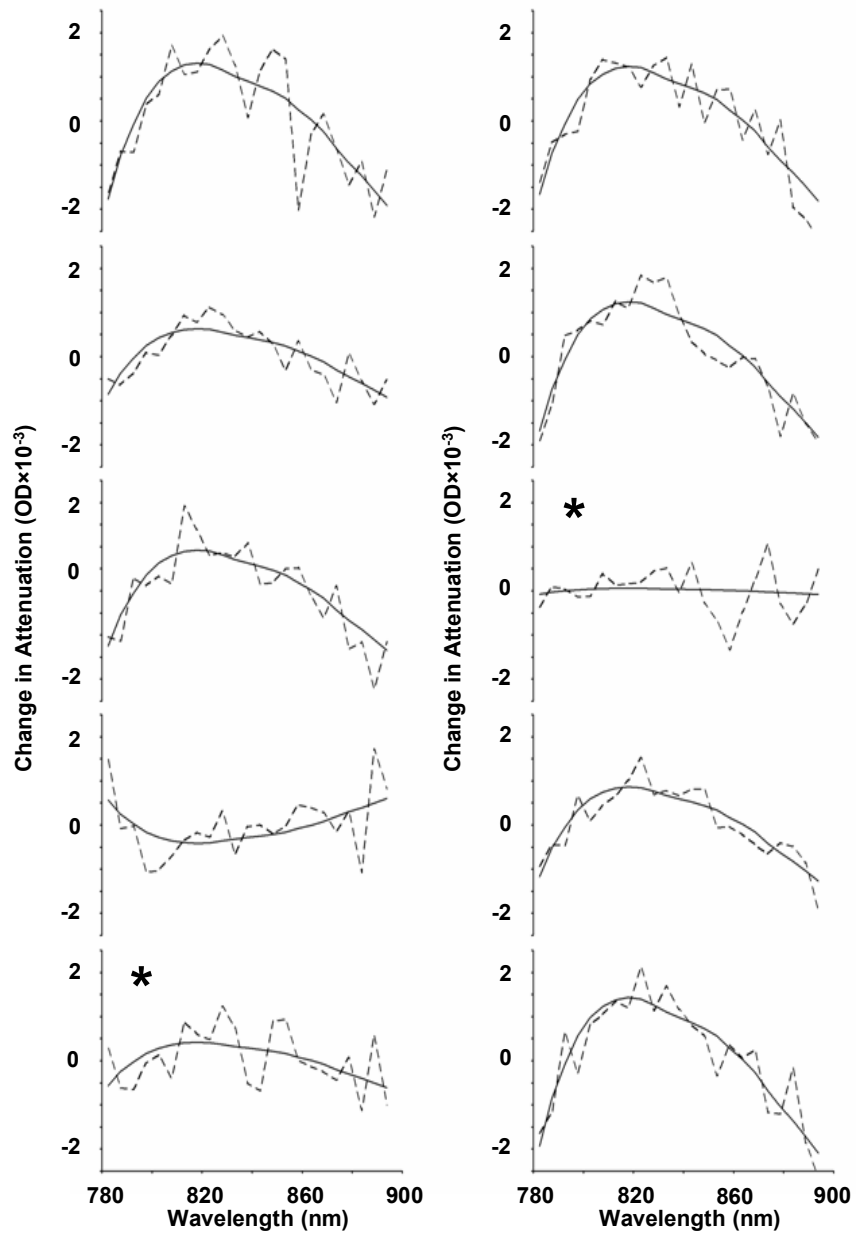


Figure 4. Residuals from 2 component fits to change in near infrared attenuation between baseline and hypoxaemia (---) and 2 component fit to simulated spectrum (—) for each individual subject. Two subjects had significant differences between the two sets of residuals (marked with *).

4. CONCLUSIONS

The quality of a multiple regression fit can be determined by assessing the residuals of the fitting procedure. The better the fit the smaller will be the sum of the square of the residuals, with the perfect theoretical fit having residuals all equal to zero. Furthermore, any residuals which are present should be randomly distributed around zero. The presence of residuals which are not randomly distributed suggests that there is a component missing from the fitting analysis.

The residuals from the 2 component fit to the group mean experimental spectrum do not appear independent and show a broad peak located around 830 nm which is similar to the oxCCO difference spectrum. This suggests that the attenuation of NIR spectra by the healthy human brain during hypoxaemia cannot be accounted for solely by $\Delta[\text{HHb}]$ and $\Delta[\text{HbO}_2]$. When the 3 component model is used, to also fit for $\Delta[\text{oxCCO}]$, the sum of the squares of the residuals is reduced and the residuals appear random, thus improving the fit. The simulated spectrum assumes that $\Delta[\text{HHb}]$, $\Delta[\text{HbO}_2]$ and $\Delta[\text{oxCCO}]$ are occurring and that no other chromophores are causing a change in optical attenuation. The residuals from the 2 component fit to this spectrum are those that would be expected from the 2 component fit to the experimental data if $\Delta[\text{HHb}]$, $\Delta[\text{HbO}_2]$ and $\Delta[\text{oxCCO}]$ were occurring in the brain during this hypoxaemic challenge. The residuals from the 2 component fit to the experimental spectrum do not differ from those resulting from the 2 component fit to the simulated spectrum. This strongly suggests that $\Delta[\text{oxCCO}]$ is occurring and this accounts for the residuals from the 2 component to the experimental data.

There is no statistical difference between the individual 2 component fits to the experimental and simulated data in eight out of ten of the individual subjects. This demonstrates the optical effect of $\Delta[\text{oxCCO}]$ at the individual as well as the group level. In the two subjects who exhibited differences between the residuals to the experimental and simulated data, the simulated residuals are very close to zero. This results in a very low dispersion in the residuals to the simulated data. It is possible that in these two individuals the physiological challenge was insufficient to produce a significant change in $\Delta[\text{oxCCO}]$.

We postulate, therefore, that changes in cerebral CCO redox state occur during moderate hypoxaemia, and that we can detect these changes using non-invasive BBS. We suggest that one should fit the oxidised-reduced CCO difference spectra when using NIRS to monitor the brain during hypoxaemia. It has been suggested that NIRS algorithms using a small number of discrete wavelengths are less capable of separating the HbO_2 , HHb and oxCCO signals¹⁰. This broadband spectroscopy dataset collected using multiple wavelengths will allow us to test various sets of wavelengths in order to determine which subsets perform best.

We are currently using BBS to study changes in human cerebral CCO redox state occurring after traumatic brain injury. This measurement may be able to provide clinically relevant information with which to guide neuroprotective treatment of acute brain injury on the neurocritical care unit.

5. ACKNOWLEDGEMENTS

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