An investigation of the relationship between BOLD and perfusion signal changes during epileptic generalised spike wave activity

David W Carmichael PhD[1], Khalid Hamandi MRCP[1,3], Helmut Laufs MD[1], John S Duncan FRCP[1], David L Thomas PhD[2], Louis Lemieux PhD[1]

Author affiliations:

1) Department of Clinical and Experimental Epilepsy, UCL Institute of Neurology, Queen Square, London and National Society for Epilepsy, Chalfont St Peter, Buckinghamshire, UK.
2) Department of Medical Physics and Bioengineering, UCL, London, UK
3) The Epilepsy Unit, University Hospital of Wales, Cardiff, UK

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Corresponding author: David Carmichael

Correspondence to:

Wellcome Trust High Field MR Research Laboratory

University College London

12 Queen Square

London WC1N 3AR

Tel +44 (0)20 76762006

Fax +44 (0)20 76762005

email d.carmichael@ion.ucl.ac.uk

Introduction

Altered brain metabolism and perfusion may confound functional imaging studies in patients with epilepsy. Generalised spike wave activity (GSW), the electroencephalographic (EEG) hallmark of absence seizures, occurs in idiopathic generalised epilepsy but can also occur in secondarily generalised patients. Some patients have GSW without any clinical manifestation and have been studied with EEG-fMRI, which characteristically shows thalamic activation and widespread cortical 'deactivations' (Salek-Haddadi et al 2003, Hamandi et al 2006, Gotmann et al 2005).

In pathological conditions interpretation of functional MRI (fMRI) results can be difficult. This is due to a reliance on the assumed coupling between neuronal activity and changes in cerebral blood flow (CBF) and oxygenation that are predominantly responsible for the signal changes seen in gradient echo images via blood oxygen level dependant contrast (BOLD) (Ogawa et al 1990, Hoge et al 1999).

We wanted to investigate the coupling between BOLD and CBF time courses in epilepsy patients with GSW to try to better understand the underlying mechanisms behind the EEG-fMRI signal changes observed especially in regions of negative BOLD response (NBR).

In a few studies this neurovascular coupling has been investigated with calibrated BOLD experiments (e.g. Hoge et al 1999, Stefanovic et al 2005). These allow simultaneous non-invasive estimation of perfusion and the rate of cerebral oxygen consumption (CMRO2). A difficulty of this method is that the investigation of these relationships relies on significant co-localised brain activation in both the BOLD and perfusion time courses and calibration with graded hypercapnia. The low signal to noise ratio (SNR) of both the BOLD and perfusion time courses (Aguirre et al 2002) is a particular problem in epilepsy studies where responses to spontaneous activity are investigated rather than a controlled task with optimised paradigms. The different SNR of these two also means that the choice of a significant threshold for each time series is highly problematic. Even in the case where significant signal changes are seen in both BOLD and perfusion time series there are issues to be overcome with regard to spatial localisation. Finally, even when this has been achieved, demonstrating coupling in one small brain area does not easily allow for generalisation over the rest of the brain.

In this paper we investigate in more detail the relationship between BOLD and CBF time courses using a simple method that does allow for observations to be made over the whole brain without the need for spatially consistent (de/) activations within the two different signals. In particular we examine the relationship between CBF and BOLD changes to see if there was a difference between rest and GSW that could indicate altered cerebral oxygen status.

Methods

Four patients with frequent GSW were scanned with simultaneous EEG-fMRI with BOLD and ASL sequences. Patient and data acquisition details can be found in Hamandi et al 2007 (Hamandi et al, *in press*).

EEG Acquisition and Processing

32 channels of surface EEG were recorded in the MR scanner using MRI compatible hardware (BrainAmp MRplus, Brainproducts, Munich, Germany; BrainCap MR, Easycap, Herrsching-Breitbrunn, Germany). Scanner and EEG clock were synchronized (Mandelkow et al., 2006) facilitating improved EEG quality after imaging and pulse artifact subtraction (Vision Analyzer, Brain Products) (Allen et al., 1998; Allen et al., 2000). The start and stop of GSW events were visually marked on the EEG according to the fMRI time series.

MRI acquisition

Imaging was carried out on a 3T Siemens Allegra head scanner (Siemens, Erlangen, Germany) using a standard head transmit/receive coil.

A 30 minute time series was obtained with a pulsed arterial spin labelling (PASL) sequence (Q2TIPS) (Luh et al., 1999, Nöth et al, 2006) with the PICORE labelling scheme. The scanning parameters were: TR 2.3 sec, (time for acquisition of a single slice was 66ms, $TI_1/TI_{1stop}/TI_2$ = 600/1200/1300ms), TE 30ms, 6 axial slices (extending superiorly from the top of the corpus callosum), 4 mm slice thickness, slice gap 0.5 mm FOV 22.4 cm x 22.4 cm, matrix 64 x 64 (see Nöth et al, 2006 for full sequence details).

MRI processing

In-house software written in Matlab (www.mathworks.com) for the calculation of perfusion images. BOLD and ASL series were pre-processed separately. Standard GLM analyses were run in SPM2 (http://www.fil.ion.ucl.ac.uk/SPM) see Hamandi et al 2007 (Hamandi et al *in press*).

ASL series: The Siemens MoCo (motion correction in frequency space before image reconstruction) series of label and control images were used for analysis. Images were further realigned to the first image using SPM2. A time series of the difference images, (control-label), was calculated by subtracting adjacent control and temporally adjusted label images; a ‘surround average’ of preceding and

following label images was used to remove effects of BOLD signal fluctuation within one TR (Aguirre et al., 2002), and expressed as a ratio of the control image to remove BOLD contrast present in both label and control images (Garraux et al., 2005) and create a relative CBF time series.

We thresholded difference images according to Garraux et al (Garraux et al., 2005). In brief, abnormal flow values that did not fall within a physiological range (e.g. due to head motion) were removed, by retaining only 1) pairs of voxels where control signal intensity had a value greater than 80% of the global mean intensity of the control image, and 2) voxels with a fractional signal change of less than $\pm$ 5%.

A corresponding BOLD sensitive time series was calculated from the ASL data by summation of adjacent label and control images.

BOLD and CBF correlation analysis

Firstly, a design matrix was created using SPM for both the ASL and BOLD time series and used to generate a time series of the confounding variance (due to motion) that was then removed by subtraction. Then linear drift was removed from the BOLD time course (using the Matlab detrend.m function). Both these time series were converted into units of %change which will be referred to as ΔBOLD and ΔCBF measurements. Pixels with a very low (e.g. white matter) or very high (e.g. edges) mean ΔCBF were removed.

To investigate the coupling between the ΔCBF and ΔBOLD measurements, we performed a voxel-wise linear regression between the two using a one way analysis of covariance (ANCOVA) as implemented in the aoctool.m function in the Matlab statistics toolbox (www.mathworks.com). An additional regressor used to assign the data at each time point to either 1) a rest epoch (images acquired during background EEG activity), or 2) a GSW epoch (images acquired during GSW). This is used to determine if there is a difference in this relationship between EEG-defined states (rest and GSW). In particular, we examined the slope of the ΔCBF and ΔBOLD on a voxelwise basis ($p<0.001$ uncorrected) and for the significant voxels the signal was averaged and correlation coefficients were obtained for rest and GSW periods.

Results

Over the whole time series, irrespective of EEG state, nearly the entire imaged brain volume showed a significant positive correlation between BOLD and CBF signal in all patients (figure 1). There was some considerable regional variation in the slope value. For each subject, the region of the brain demonstrating a significant correlation was used to obtain an average time course. This was then investigated to obtain an average relationship for ΔCBF/ΔBOLD (Table 1). The range of values lay between +19 and +36 and was highly significant in all subjects. It should be noted that this is despite widespread cortical NBR seen by standard GLM analysis (Hamandi et al, 2007). There was not a significant difference in this relationship for the two EEG-defined states (rest and GSW) either at a voxel or brain-average level ($p<0.01$) and the sign of the change varied.

Discussion

BOLD and CBF were positively correlated in patients with epilepsy during background EEG activity and GSW. Interestingly, the value of the ΔCBF/ΔBOLD slope did seem to show spatial variation which could indicate areas with altered vascular response as has been seen in hypoxia (Nöth et al, 2007) or regions with different ranges of oxygen consumption. Significant differences in ΔCBF/ΔBOLD were not found due to GSW compared to background changes in spontaneous activity.

Pre-processing of the data followed by the analysis presented could introduce some level of correlation into the data and/or alter their statistical significance by reducing the apparent degrees of freedom. However, the analysis was run without removing motion confounds and similar results were obtained. We assumed a linear relationship between ΔCBF and ΔBOLD which is a reasonable approximation over the likely range of ΔCBF (Hoge et al, 1999). Additionally, we chose a relatively high level of significance ($p<0.001$ uncorrected) as a threshold for ΔCBF/ΔBOLD. Significant differences in ΔCBF/ΔBOLD between rest and GSW were not found. The values of the ΔCBF/ΔBOLD slope are in broad agreement with those obtained in cognitive paradigms in healthy subjects (Hoge et al, 1999, Stefanovic et al, 2005) where changes in oxygen consumption make the ΔCBF/ΔBOLD gradient higher than would be expected for changes in CBF alone (as for hypercapnia). This indicates that neurovascular coupling and cerebral energy consumption is not altered between states, or in regions exhibiting deactivations. While we do not have a direct measure of neural activity or oxygen consumption, because we know GSW is associated with cortical inhibition, we infer that this inhibition lowers metabolic demands reflected in a reduction in perfusion that leads to a reduction in the BOLD signal, in a proportion suggesting that neurovascular coupling to the BOLD signal is maintained as in the experimental studies of Shmuel et al (Shmuel et al, 2006). However, we cannot exclude the possibility that differences were below a detectable level.

In previous work, the examination of BOLD and perfusion time courses have relied on analysing spatially overlapping (de/) activations in both BOLD and perfusion time courses (Stefanovic et al, 2005). This is problematic due to the differing sensitivity of the two methods (Aguirre et al, 2002) and presents a particular difficulty when the task is not controlled – as for spontaneous epileptic discharges. In brain areas that do show coincident BOLD and perfusion positive or negative responses it is highly likely that normal neurovascular coupling will be present. We have adopted a simple

approach of looking at the correlation of the two signals on a voxel-by-voxel basis to examine this relationship in areas where BOLD and perfusion do not necessarily both show coincident activation.

For regional assessment we note that constructing a full model containing both confounds and the perfusion time course for comparison to the BOLD signal is preferred for the most accurate statistical determination of the strength of this relationship. However, for voxel by voxel analysis this would require a different 'design matrix' to be used for each voxel.

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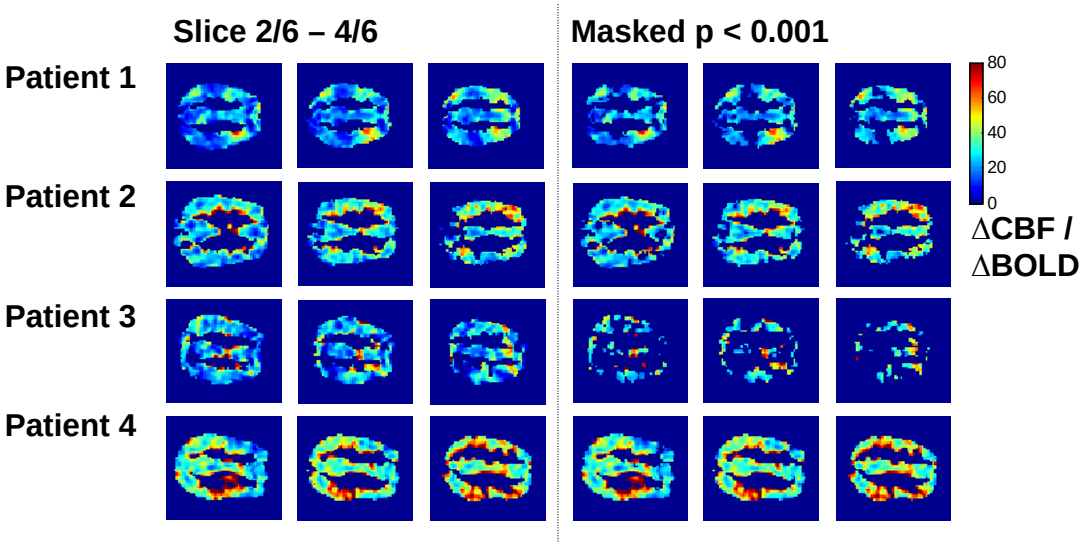

Figure 1 a comparison of the percentage change in CBF to the percentage change in BOLD signal for 4 patients (each row). The ratio of ΔCBF/ΔBOLD (i.e. the slope of the linear fit between the two signal changes) is shown for each voxel on the left hand side. On the right hand side voxels are thresholded at a level of p<0.001 uncorrected) the average time signal from these voxels was averaged and tested (see Table 1).

Patient	ΔCBF / ΔBOLD all	p	ΔCBF / ΔBOLD rest	ΔCBF / ΔBOLD GSW	p
1	19.3	<0.001	18.1	20.5	0.772
2	22.3	<0.001	11.3	33.3	0.042
3	29.1	<0.001	30.7	27.5	0.805
4	36.5	<0.001	44.2	28.8	0.035

Table 1 The average slope of ΔCBF / ΔBOLD signal changes taken over all significant voxels for each patient assessed over the whole time series and then for differences between rest and GSW.