

FALSE POSITIVES IN FUNCTIONAL NEAR- INFRARED TOPOGRAPHY

Ilias Tachtsidis¹, Terence S. Leung¹, Anchal Chopra¹, Peck H. Koh¹,
Caroline B. Reid¹, and Clare E. Elwell¹

Abstract: Functional cranial near-infrared spectroscopy (NIRS) has been widely used to investigate the haemodynamic changes which occur in response to functional activation. The technique exploits the different absorption spectra of oxy- and deoxy-haemoglobin ($[\text{HbO}_2]$ $[\text{HHb}]$) in the near-infrared region to measure the changes in oxygenation and haemodynamics in the cortical tissue. The aim of this study was to use an optical topography system to produce topographic maps of the haemodynamic response of both frontal cortex (FC) and motor cortex (MC) during anagram solving while simultaneously monitoring the systemic physiology (mean blood pressure, heart rate, scalp flux). A total of 22 young healthy adults were studied. The activation paradigm comprised of 4-, 6- and 8- letter anagrams. 12 channels of the optical topography system were positioned over the FC and 12 channels over the MC. During the task 12 subjects demonstrated a significant change in at least one systemic variable ($p \leq 0.05$). Statistical analysis of task-related changes in $[\text{HbO}_2]$ and $[\text{HHb}]$, based on a Student's t-test was insufficient to distinguish between cortical haemodynamic activation and systemic interference. This lead to false positive haemodynamic maps of activation. It is therefore necessary to use statistical testing that incorporates the systemic changes that occur during brain activation.

1. INTRODUCTION

When analysing cerebral haemodynamic activation data using functional neuroimaging the task-specific activation observed is due to the existence of a close coupling between regional changes in neuronal activation, brain tissue metabolism and regional changes in cerebral blood flow (CBF). Cranial functional near-infrared spectroscopy (NIRS) has been widely used to investigate the haemodynamic changes, which occur in response to functional activation of specific regions of the cerebral cortex. The technique exploits the different absorption spectra of oxy-haemoglobin (HbO_2) and

¹ Department of Medical Physics and Bioengineering, Malet Place Engineering Building, University College London, Gower Street, London WC1E 6BT, UK

deoxy-haemoglobin (HHb) in the near-infrared region to measure the changes in oxygenation and haemodynamics in the brain cortical tissue. In order for this response to be monitored unambiguously it is important that the haemodynamic task-related activity is occurring on top of an unchanged global systemic and brain resting state.

We have previously reported that significant changes in mean blood pressure (MBP) and heart rate (HR) occur during anagram activation tasks and observed that NIRS haemodynamic changes were in some volunteers significantly correlated with changes in these systemic variables.¹ Most recently,² we reported that during a frontal lobe anagram activation task, task-related haemodynamic changes were observed both over the frontal cortex (activated region) and motor cortex (control region). The task-related changes were correlated with increases in MBP and scalp blood flow (flux) measured with laser Doppler. This implies the possibility of some systemic “global interference” in our NIRS measured data. It is possible that the anagram task elicits an emotional response, which produces changes in blood pressure that are likely to cause passive changes in the scalp blood flow. These changes can produce small task-related, but non cortical alterations in the [HbO₂] and [HHb] signals as measured by cranial NIRS.

Over the last decade or so, many studies have been published describing the use of the optical topography (OT) technique to map functional brain activation.³⁻⁵ By making simultaneous NIRS measurements at multiple brain sites, one can produce spatial maps of the haemoglobin concentration changes that correspond to specific regions of the cerebral cortex. OT can therefore potentially discriminate between regional activated cortical areas and global haemodynamic changes.

The aim of this study is to investigate the functional haemodynamic changes during frontal lobe anagram activation using optical topography both over the activated and control area while continuously monitoring systemic and scalp blood flow changes.

2. MATERIAL AND METHODS

This study was approved by the UCL Research Ethics Committee. We studied 22 young healthy subjects with English as their first language (15 male, 7 female, median age 22 years, range 20-39).

NIRS measurements were conducted with the ETG-100 Optical Topography System (Hitachi Medical Co., Japan) using two 12-channel arrays. Each optode array consisted of 5 source optodes (each delivering light at 780 and 830 nm) and 4 detector optodes. The source-detector interoptode spacing was 30mm and data were acquired at 10Hz. The optodes were placed over the subject's left frontal cortex and positioned according to the international 10-20 system of electrode placement such that channels 1-12 were centred approximately over the frontopolar region (Fp) and channels 13-24 were centred approximately over the left primary motor cortex (C3). A schematic illustration of optode placement is shown in Figure 1.

A Portapres® system (TNO Institute of Applied Physics) was used to continuously and non-invasively measure MBP and HR from the finger. A laser Doppler probe (FloLab, Moore Instruments) was placed over the forehead to monitor the changes in scalp blood flow (flux).

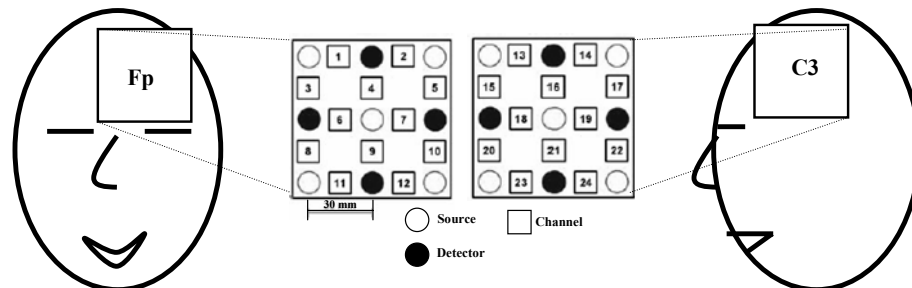


Figure 1. A schematic diagram illustrating the approximate positions of the NIRS light sources, detectors and locations of corresponding measuring positions/channels. One array was centred on the frontopolar region (Fp), the other on the left motor cortex (C3).

All the volunteers were positioned in a comfortable sitting position. Data were recorded during two minutes of the subject at rest (baseline), followed by 45 seconds of the subject solving 4-letter anagrams (9 anagrams, 5 seconds per anagram), 30 seconds rest, 45 seconds of solving 6-letter anagrams (5 anagrams, 9 seconds per anagram), 30 seconds of rest, 45 seconds of solving 8-letter anagram (5 anagrams, 9 seconds per anagram) and 30 seconds of rest. Each anagram-solving period was repeated a total of four times, with the study ending after a 2-minute rest period (total study time 19 minutes). In this study solving an anagram was defined as producing one coherent word using only the letters from another word (e.g. icon – coin). Subjects were encouraged to solve as many anagrams as possible and were instructed to say possible solutions out loud (without moving).

All optical data were subjected to an identical processing procedure using the functional Optical Signal Analysis program⁶ (fOSA, University College London, UK) to convert the relative changes in light intensities to concentration changes in haemoglobin (HbO_2 , HHb and their sum, HbT) using a differential pathlength factor correction of 6.26. All the signals including MBP, HR and flux, were then decimated from 10Hz to 1Hz and low pass filtered at 0.08Hz. The data were filtered using a 5th order low pass Butterworth digital filter in forward backward directions to avoid introducing a phase delay. The last pre-processing stage, prior to statistical analysis was to de-trend the time-course to remove both drift introduced by the system and any slowly changing unrelated physiological signals. A first-order linear baseline was drawn as the reference and then subsequently subtracted from the activation signal.

The response to stimulation was calculated for each subject as the difference between the average of 10 seconds worth of baseline data at the end of the rest period, and the average of 10 seconds of data commencing 15 seconds after the onset of the 4, 6 or 8 letter anagram solving periods respectively. A ‘Student’s t-test’ was used to assess the significance of these responses (the threshold of significance was set at $p \leq 0.05$ from baseline). For the optical topographic data we then calculated the cumulative total number of channels across subjects in which we observed activation. We define activation as a statistical significant increase in $[\text{HbO}_2]$, a statistical significant decrease or no change in $[\text{HHb}]$ and a statistical significant increase in $[\text{HbT}]$. Systemic interference was measured by using the Pearson correlation model to calculate correlations between the systemic variables and changes in $[\text{HbO}_2]$ and $[\text{HHb}]$ in all of the OT channels.

3. RESULTS

A summary of the activation data for the whole group is shown in Figure 2. Each paradigm is shown separately and data are normalised to the number of valid channels. Across paradigms similar activation response was observed in both frontal cortex and motor cortex. Channels in which the highest number of subjects showed activation were channel 23 (55.56%) for the 4-letter task, channel 1 (52.94%) for the 6-letter task, and channels 6 and 21 (33.33%) for the 8-letter task. Taking into account all of the tasks, an average of 30% of the subjects showed activation (range 25-35%) on the frontal cortex and 27% (range 17-37%) on the motor cortex.

Analysis of the systemic variables show that at least 50% of the subjects demonstrated a change in at least one systemic variable. Table 1 shows the mean changes in each systemic variable for those subjects that showed a significant change.

Correlation analysis of the NIRS and systemic data shows a large variability across different OT channels and across subjects. Figure 3 shows the results of the correlation analysis between MBP and the NIRS data, across all channels for (a) subject 3 who showed generally high correlations ($r > 0.5$), and (b) subject 18 who showed generally low correlations ($r < 0.5$). Both subjects showed significant changes in systemic variables during the anagram tasks and both subjects had channels that showed activation. This trend was observed across subjects. The correlation between the systemic data and the NIRS data from the frontal cortex channels show no difference from the correlation between the systemic data and the NIRS data from the motor cortex channels.

Table 1. Group changes from rest to activation are presented as mean \pm standard deviation for those subjects that demonstrated a significant change.

Systemic Variables	4-letter task	6-letter task	8-letter task
Δ [MBP] (mmHg)	(n=11) 6.9 \pm 2.7	(n=12) 6.3 \pm 4.9	(n=12) 6.9 \pm 1.9
Δ [HR] (beats/min)	(n=5) 4.3 \pm 4.9	(n=6) -0.4 \pm 6	(n=6) 2.4 \pm 4.3
Δ [Flux] (%)	(n=4) 14.3 \pm 31.1	(n=3) 20.3 \pm 10.3	(n=1) -17.8

4. DISCUSSION

In this study we used an optical topography system to investigate the changes in [HbO₂] and [HHb] during anagram solving over the frontal lobe (activated area) and motor cortex (control area) while simultaneously monitoring systemic variables. We used a Student's t-test to define significant changes in [HbO₂], [HHb] and [HbT] for each OT channel and for each subject during the different anagram solving tasks and used these data to define where and when activation was detected. The same analysis was performed on the systemic variables. We observed a large variability in activated OT channels across subjects. The OT results failed to define specific regional areas of activation. 50% of subjects showed a significant change in at least one systemic variable. These systemic changes appear in some subjects to correlate with the observed functional changes in [HbO₂] and [HHb] across the OT channels. Figure 4 shows an example of changes in [HbO₂] and [HHb] from an OT channel over the frontal cortex and an OT channel over the motor cortex with the simultaneously recorded changes in MBP and scalp flux.

Clearly systemic interference during the anagram task can lead to false positives in defining activated OT channels.

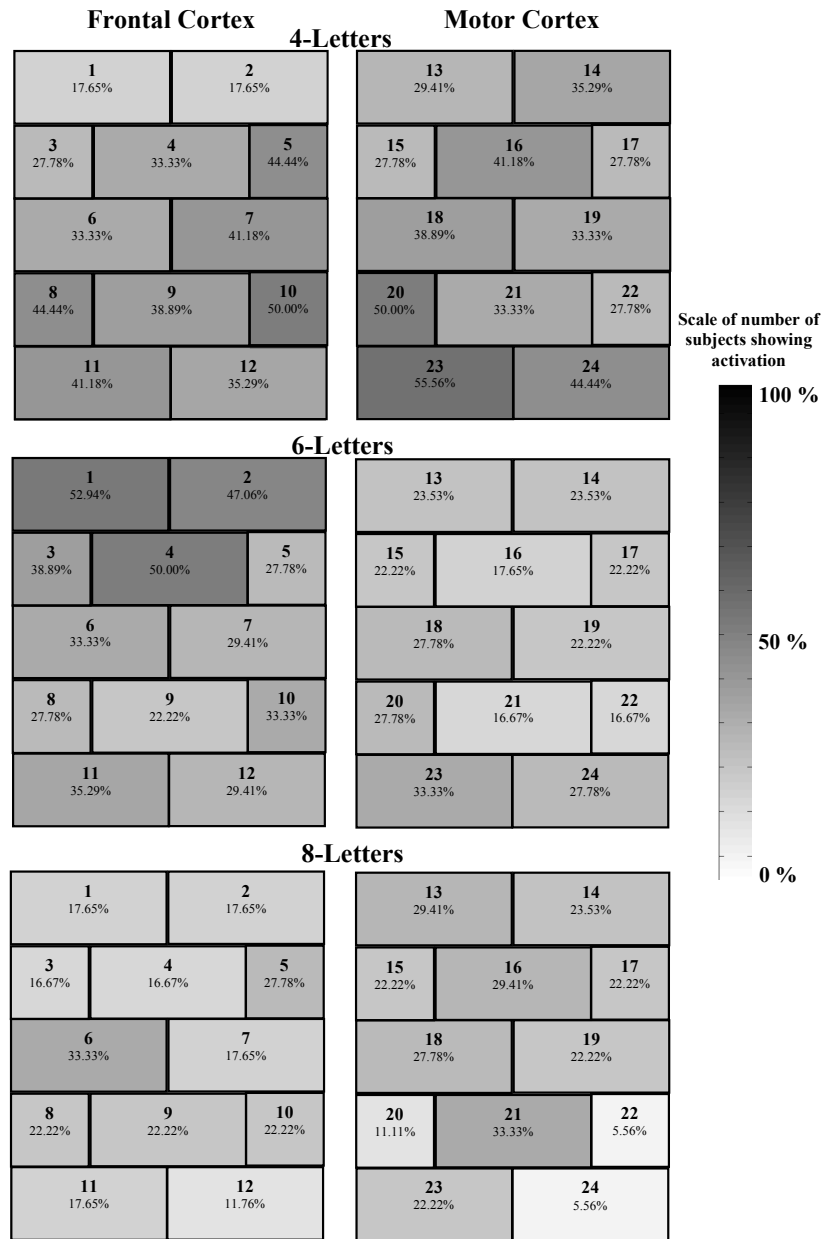


Figure 2. Group analysis shows the percentage of subjects that demonstrated activation in specific channels during the three different anagram solving paradigms.

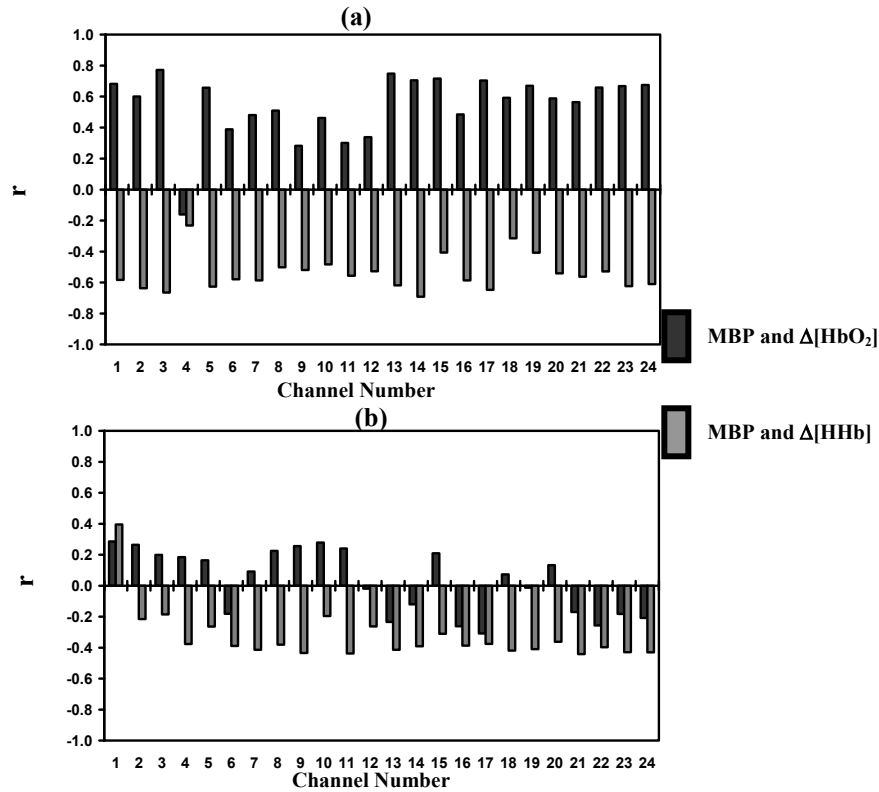


Figure 3. Individual correlation coefficients between MBP and $\Delta[\text{HbO}_2]$ and MBP and $\Delta[\text{HHb}]$ across all channels for (a) subject 3 and (b) subject 18.

In this study we used the classical approach to define significant changes in haemoglobin concentrations by employing a “Student’s t-test”. This approach compares two different states of the brain, i.e. “rest” versus “activation”. The “rest” period is usually defined as a baseline period before the stimulus onset and the “activation” period is defined as the period 10-20 seconds after the onset of the stimulus. By keeping the rest and activation periods constant across subjects one can investigate the functional response to specific tasks. Whilst a simplistic approach of this kind helps to provide a quick assessment of the haemodynamic response to the task it does not consider any spatial coherence in the OT data. It also assumes that the measured changes in haemoglobin concentrations are due solely to the neuronal activation, and that there are no task-related systemic effects. We have shown that this latter assumption is not true for all subjects performing an anagram solving task. One can include a priori information regarding systemic changes and can de-correlate the physiological noise (cardiac, respiratory and vasomotion related fluctuations) from the evoked haemodynamic response, by using techniques such as Principal Component Analysis,⁷ Independent Component Analysis,⁸ and more recently Statistical Parametric Mapping (SPM).⁶ SPM has been widely used for the analysis of functional activation data from other neuroimaging modalities such as the BOLD response in fMRI studies.⁹ SPM uses mass

univariate approach to modelling the spatiotemporal neuroimaging data by assigning a statistic value to every brain voxel. It enables the construction of spatial statistical processes to test hypotheses about regional specific effects in the brain. Unlike the classical approach mentioned earlier, where the two different time courses compared, SPM employs a modelling approach for each brain voxel. In our study all of the explanatory variables (HbO_2 , MBP, HR and flux) were treated as regressors in the linear model. To treat the variability of haemodynamic responses arising from different events between different brain voxels, SPM allows the modelling of latency and dispersion derivatives as additional regressors to its canonical response function. The associated parameter estimates are the coefficients for each of the regressors that best model the observed response for the voxel in question (here a voxel is defined as an OT channel). To account for the spatial coherence of the functional data, SPM provides the necessary family-wise correction based on the theory of Gaussian random field to resolve the multiple comparison problem.

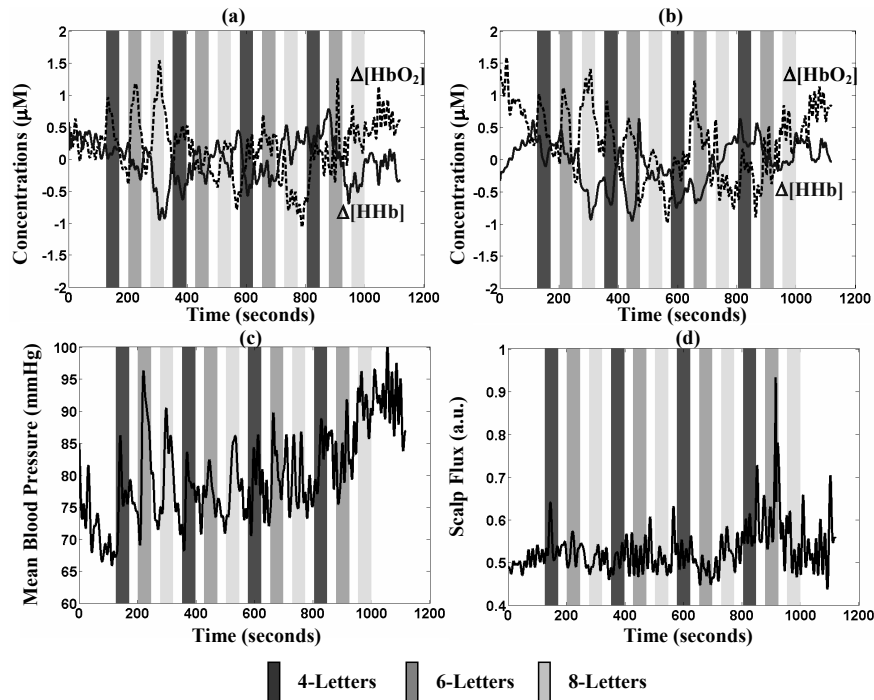


Figure 4. Results from one subject showing changes in; (a) NIRS data from channel 3 (frontal cortex) (b) NIRS data from channel 15 (motor cortex); (c) MBP and (d) scalp flux.

As an example of this method we have used fOSA-SPM software⁶ to analyse NIRS and systemic data from one subject collected during the 6-letter anagram solving task. Using the “Student’s t-test” analysis, this subject demonstrated activation across all OT channels. Figure 5 shows the results of the SPM analysis on the same subject’s data. These are presented as an SPM t-result for the HbO_2 signal over all channels and show a spatial localisation of the haemodynamic response. Unlike the “Student’s t-test” approach which compares the difference between two specific physiological states, SPM offers a more rigorous approach to analysing functional OT data by taking into account the global

systemic effects by means of fitting a haemodynamic response function and performing spatial correlations across all channels.

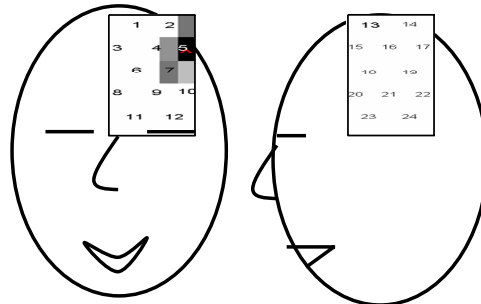


Figure 5. The SPM t-results with a threshold of significance of $p \leq 0.05$; darker pixels correspond to higher significant t-values.

In conclusion, when analysing OT data for evidence of functional activation the effect of task-related changes in systemic variables should be taken into account. SPM may be a useful tool for analysing simultaneously measured multi-channel OT NIRS data and systemic variables.

5. ACKNOWLEDGMENTS

The authors would like to acknowledge the EPSRC (Grant No EP/D060982/1).

6. REFERENCES

1. I. Tachtsidis, T.S. Leung, L. Devoto, D.T. Delpy, and C.E. Elwell, Measurement of frontal lobe functional activation and related systemic effects: a near-infrared spectroscopy investigation, *Adv. Exp. Med. Biol.* In Press (2008).
2. I. Tachtsidis, T.S. Leung, M.M. Tisdall, D. Presheena, M. Smith, D.T. Delpy, and C.E. Elwell, Investigation of frontal cortex, motor cortex and systemic haemodynamic changes during anagram solving, *Adv. Exp. Med. Biol.* In Press (2008).
3. Y. Hoshi, B. H. Tsou, V. A. Billock, M. Tanosaki, Y. Iguchi, M. Shimada, T. Shinba, Y. Yamada, and I. Oda, Spatiotemporal characteristics of hemodynamic changes in the human lateral prefrontal cortex during working memory tasks, *NeuroImage* **20**(3), 1493-1504 (2003).
4. B. Chance, S. Nioka, S. Sadi, and C. Li, Oxygenation and blood concentration changes in human subject prefrontal activation by anagram solutions, *Adv. Exp. Med. Biol.* **510**, 397-401 (2003).
5. R.P. Kennan, D. Kim, A. Maki, H. Koizumi, and R.T. Constable, Non-invasive assessment of language lateralization by transcranial near infrared optical topography and functional MRI, *Hum. Brain Mapp.* **16**(3), 183-189 (2002).
6. P.H. Koh, D.E. Glaser, G. Flandin, S. Kiebel, B. Butterworth, A. Maki, D.T. Delpy, and C.E. Elwell, Functional optical signal analysis (fOSA): a software tool for NIRS data processing incorporating statistical parametric mapping (SPM), *JBO* In Press (2007).
7. X. Zhang, V. Toronov, and A. Webb, Simultaneous integrated diffuse optical tomography and functional magnetic resonance imaging of the human brain, *Opt. Express* **13**(14), 5513-5521 (2005).
8. I. Schiessl, M. Stetter, J.E.W. Mayhew, N. McLoughlin, J.S. Lund, and K. Obermayer, Blind signal separation from optical imaging recordings with extended spatial decorrelation, *IEEE Transactions on Biomedical Engineering*, **47**(5), 573-577 (2000).
9. K.J. Friston, A.P. Holmes, J.B. Poline, P.J. Grasby, S.C. Williams, R.S. Frackowiak, and R. Turner, Analysis of fMRI time-series revisited, *NeuroImage* **2**(1), 45-53 (1995).