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Coupling of fMRI and NIRS measurements in the study of negative BOLD response to intermittent photic stimulation*

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***Abstract*— Functional Magnetic Resonance Imaging (fMRI) in combination with Near Infrared Spectroscopy (NIRS) is finding widespread use in the analysis of brain function. While most of the studies deal with the detection of positive responses, here we focus on negative responses to visual stimulation. In a group fMRI study on Intermittent Photic Stimulation (IPS) we detected a sustained Negative BOLD Response (NBR) in the extrastriate visual cortex. To confirm and better characterize NBR, we repeated the same protocol during NIRS recordings. In this paper we show fMRI results and demonstrate the NBR on the basis of NIRS findings.**

I. Introduction

Intermittent Photic Stimulation (IPS) stimulates the retinae and visual cortex through multiple intermittent flashes at different frequencies. IPS is commonly used during ElectroEncephaloGraphic (EEG) recordings to detect photosensitivity, i.e. an abnormal epileptogenic sensitivity to flickering light [1]. Several EEG works examined the effects of IPS on neuroelectrical activity, but few functional Magnetic Resonance Imaging (fMRI) studies focused on the hemodynamic modifications induced by IPS [2].

In the present paper we investigate the pattern of fMRI Blood Oxygen Level Dependent (BOLD) response to IPS in a cohort of normal subjects. While most of the studies analyze positive changes during different tasks, here we focus on Negative BOLD Responses (NBRs) detected during flashing lights stimulation. The fMRI technique is sensitive to local changes in paramagnetic deoxyhemoglobin (HHb) concentration through the BOLD contrast and, thanks to its high spatial resolution, it has become the routine method for functional brain imaging. However, this method does not quantitatively measure deoxyhemoglobin concentration [HHb], neither it provides any information on oxyhemoglobin (HbO_2) dynamics. To better characterize NBR properties, the fMRI findings are combined with those obtained from another neuroimaging technique: Near-Infrared Spectroscopy (NIRS). NIRS is an optical technique which measures the concentration of the two species (HHb and HbO_2) on the basis of the differences in their absorbing spectra. Therefore, NIRS was used to give insight into the BOLD physiological mechanisms [3-6].

In this paper, we show the fMRI results obtained from an IPS protocol administered to the healthy group. The findings are discussed and interpreted, also on the basis of NIRS ones.

II. Materials and Methods

A. Subjects

Participants were five healthy volunteers (2 males, mean age 28.8 +/- 3.8 years) with normal vision and negative history for epileptic seizures. The protocol was approved by the local Ethic Committee and a written informed consent to the study was obtained from each subject.

B. Visual stimulation protocol

The stimulation protocol was developed with the Presentation® software (Neurobehavioral Systems Inc.). Visual stimuli were delivered through MRI-compatible goggles (Resonance Technology Inc.) in the fMRI environment, and by using a computer screen in NIRS setting, respectively. The visual experiment consisted in a block-designed IPS. IPS was created by reversing black and white screens at four different frequencies (6, 8, 10 and 12 Hz). Rest epochs lasting 7 scans (14 s) were followed by stimuli epochs of the same duration. Subjects kept their eyes open during all the experiment and during rest periods they were asked to look at a yellow fixation cross in the center of a black background. Each frequency block was repeated five times in fMRI recordings, eight times in NIRS ones. As the NIRS measurements are more affected by noise, we chose a major number of repetition blocks to increase the SNR of the resulting curves. The fMRI experiment had a total duration of 580 s, the NIRS one of 928 s.

C. fMRI data acquisition

All MR exams were performed on a Philips Achieva 3 Tesla scanner (Best, The Netherlands), equipped with a 32 channels head coil. Within the MRI experiment, an anatomical T1-weighted 3D Turbo Field Echo (TFE)

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sequence was run for morphological referencing of fMRI data (1 mm isotropic resolution, Field Of View (FOV)=240x240x175 mm^3, Repetition Time (TR)=8.19 ms, Echo Time (TE)=3.74 ms, flip angle=8°). fMRI data were acquired with a T2*-weighted Gradient-Echo planar sequence (TR=2 s, TE=35 ms, flip angle=85°, 30 axial slices without gap, FOV=240x105x240 mm^3, voxel size=1.875x1.875x3.5 mm^3) covering cerebral hemispheres and excluding cerebellum and brainstem.

D. NIRS data acquisition

For the NIRS acquisition we used a commercial continuous wave NIRS device (DYNOT Compact, CE marked). An elastic cap of proper head size was fitted on the subjects' head. The cap had been previously tailored for a bilateral 16 channels montage, centered over the parieto-occipital brain area. NIRS recordings were performed at two different wavelengths (760nm and 830nm), in order to selectively probe oxygenated and deoxygenated haemoglobin species (HbO_2 and HHb respectively) in the brain.

E. NIRS channel localization

After each NIRS experiment, a structural MR sequence was acquired to provide a spatial prior for NIRS and to identify the injectors and detectors position. The localization was performed after removal of the optical probes, by attaching vitamin E pills (showing bright signal on T1-weighted MR sequences) to the NIRS cap in place of each probe. For the coregistration we used a T1-weighted 3D TFE sequence (1mm isotropic resolution, FOV=240x240x175 mm^3, TR=8.13 ms, TE=3.73 ms, flip angle=8°).

III. Data Analysis

A. fMRI data processing

The fMRI data processing was performed using SPM software (http://www.fil.ion.ucl.ac.uk/spm/, version 8). The pre-processing phase included spatial realignment for controlling head motion artifacts, coregistration to the structural images, normalization to the MNI template and spatial smoothing with a 3D Gaussian kernel filter with Full Half Width Maximum (FHWM) of 6 mm^3. We constructed a unique design matrix including the 5 subjects and all tasks, from which a fixed-effects group General Linear Model (GLM) analysis was performed. Each frequency block was taken as regressor of interest and convolved with the canonical Hemodynamic Response Function (HRF). We analyzed the effects of IPS by comparing visual stimulation with rest conditions and inference was based on a two sided t-test. The Regions Of Interest (ROIs) coordinates were saved with the SPM Marsbar toolbox (http://marsbar.sourceforge.net/). Then, the GMAC toolbox (http://selene.bioing.polimi.it/BBBlab/GMAC) was used to save the ROIs fMRI time series, which were extracted after removal of the "nuisance variables" [7]. For visualization purposes, we performed a 3D reconstruction of one subject's cortical surfaces using the Freesurfer software (http://surfer.nmr.mgh.harvard.edu/). The group contrast map was then projected onto the subject's pial surface.

B. NIRS data processing

NIRS data were visually inspected for artefact removal, and then filtered with low pass filter at 0.300 Hz. Continuous tracks were then segmented into epochs starting at the beginning of each task block, and ending 14 s after the end of the blocks. In doing so, 32 epochs, lasting 28 s each, were extracted. Epochs were grouped, in order to obtain a grand average. After a GLM analysis, t-statistics images were calculated for the IPS condition.

C. Optodes registration to the cortical surface

Source and detectors positions on the scalp were identified by means of the vitamin E markers. The coordinates of each NIRS channel were then estimated as the mean point between the corresponding injector and detector. The channels coordinates were then projected from the scalp on the cortical surface using Freesurfer.

IV. Results

A. fMRI group analysis results

The group fixed effects analysis results are shown in Fig.1. The statistical map was projected on the pial surface of one subject and corrected for multiple comparisons (False Discovery Rate (FDR) correction, $p<10^{-4}$). As expected, IPS elicited a significant PBR in the primary visual cortex of both hemispheres; the active area included calcarine and cuneal cortex, occipital pole and lingual gyrus. A major finding was the presence of two large symmetric regions with a significant negative response to IPS. The NBRs were located in extrastriate visual cortex, mostly in lateral occipital area. The ROIs showing NBRs were adjacent but spatially segregated from the main PBR.

Figure 1.

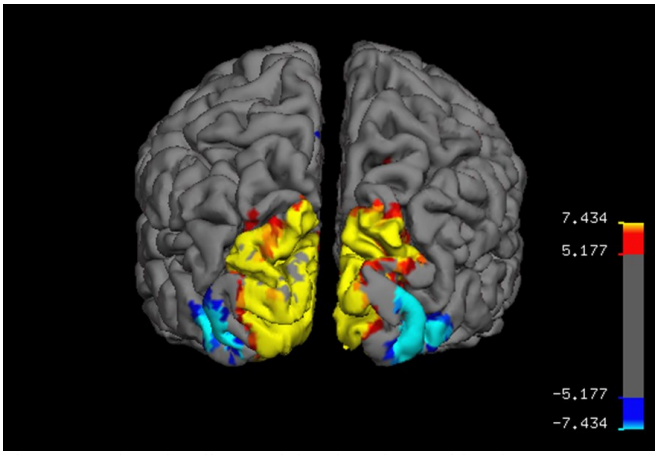

Figure 1. Activated (yellow) and deactivated areas (blue) in response to IPS resulting from the GLM group fixed effects analysis.

The amplitudes of the BOLD signal did not have remarkable differences at the different frequencies. The two symmetric NBRs had very similar time courses (Fig. 2A), therefore we considered them as a unique NBR response. The mean BOLD response to IPS is plotted in Fig. 2B. Comparing the NBR and PBR overall signal time courses, it emerged that the NBR had a lower amplitude than PBR.

During the stimulation blocks, the NBR descent was slower with respect to the PBR rise, with delayed onset time and time to peak. At the end of the IPS, the NBR return to baseline preceded the PBR one.

FIGURE 2.

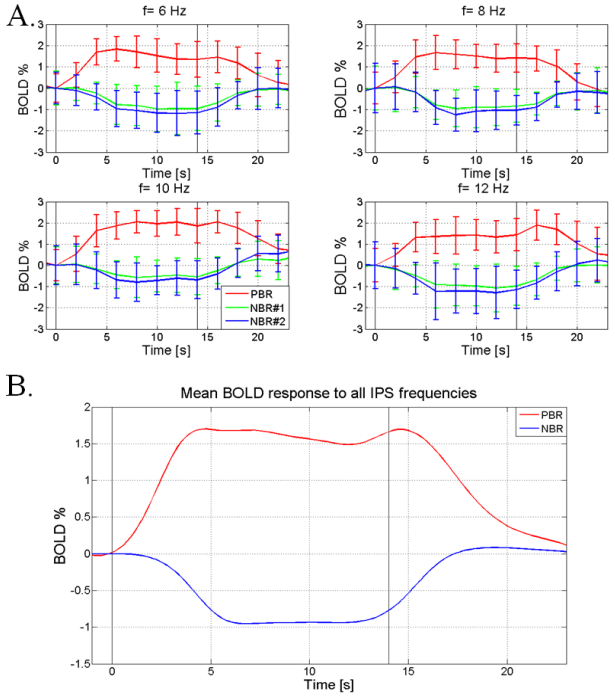

Figure 2. A. Averaging plots of the BOLD signal percent change in response to IPS blocks at 6 Hz (up left), 8 Hz (up right), 10 Hz (bottom left) and 12 Hz (bottom right). The red curves are the PBR mean time series. The green and blue curves are the mean time series of left and right NBRs respectively. B. Average BOLD response across all the IPS frequencies. The red curve is relative to PBR, the blue one is relative to the mean NBR.

B. NIRS findings

In all the five subjects who were examined, NIRS provided evidence of inversion of the canonical hemodynamic response: every subject clearly showed a response characterized by a negative [HbO_2] peak, synchronous with positive [HHb] peak and concurrent with the stimulation periods of IPS protocol in at least one channel. In four out of five cases, this pattern was glaringly bilateral. In one case the pattern was clearly detectable over the right hemisphere only, while controversial results were observed over the left hemisphere. In three subjects, the amplitude of [HbO_2] peak was definitely larger than that of [HHb] peak in at least four different channels each. These [HbO_2] amplitudes ranged from double to three times the amplitude of the correspondent [HHb] peak. GLM analysis provided further confirmation of the observed response, by showing high modules of the t-statistics (T>5 for at least one channel, in four subjects). Last, in four out of five subjects we could identify at least one channel showing non-inverted response, i.e. recording one positive [HbO_2] peak synchronous with one negative [HHb] peak, located in the primary visual cortex. The [HbO_2] and [HHb] curves of one subject are shown in Fig. 3A. Although some channels had a low SNR, an inverted activation pattern was visible in most of them; the hemodynamic inversion was confirmed by the t-statistics (Fig. 3B) with very high modules in 1-4, 3-4 and 4-8 channels ($p<0.05$). The estimated positions of the subject's NIRS channels are shown in Fig.4.

FIGURE 3.

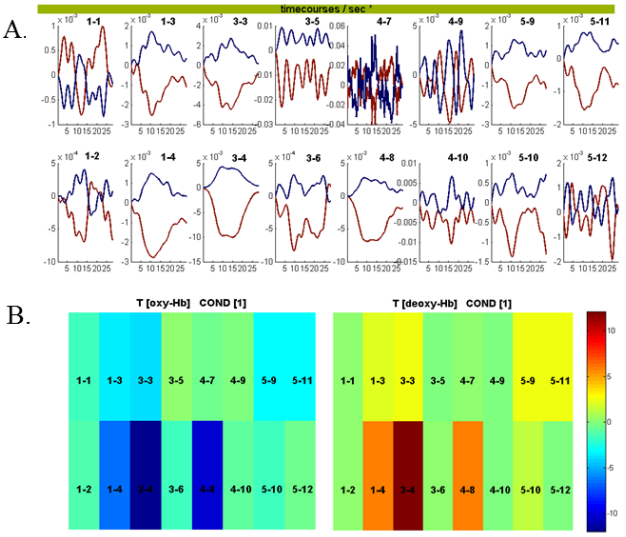

Figure 3. NIRS results relative to one subject. A. [HbO_2] (red) and [HHb] (blue) curves of the 16 channels. B. Channels T-statistics relative to [HbO_2] on the left side and to [HHb] on the right side.

FIGURE 4.

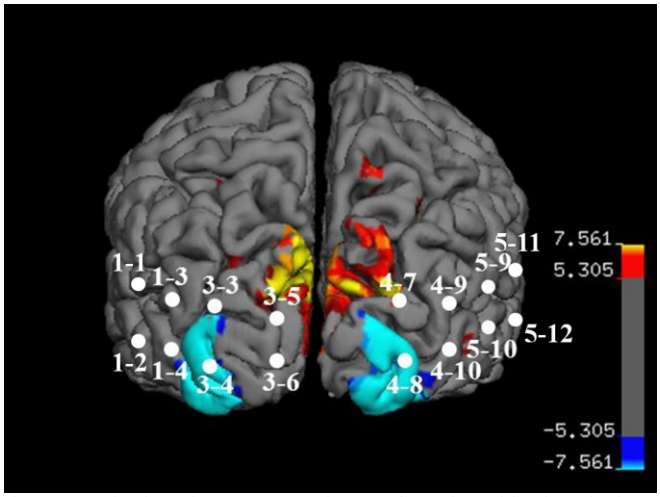

Figure 4. Projection of PBR, NBR and NIRS channels position in a single subject (same subject of Fig.3).

V. DISCUSSION

This fMRI study revealed the presence of two symmetric regions in extrastriate visual cortex with a significant negative BOLD response to IPS. As expected, we also found a strong positive response in the primary visual cortex. We therefore compared the NBR and PBR time courses, with the aim of interpreting the negative BOLD phenomenon.

The PBR and NBR regions were near but segregated; the NBR was more lateral with respect to PBR. We also detected

differences in the PBR and NBR time series with respect to time to peak, curves slope and return to baseline. While PBR was faster in reaching the peak (1s before with respect to NBR), the NBR had an earlier falling edge (before the stimulation offset) and a faster return to baseline. In particular, NBR decreased in amplitude exactly at the end of the stimulation block, while the PBR curve had an unexpected slight increase immediately after the end of IPS block. These findings were valid for IPS in general, because the NBR and PBR time courses had a good reproducibility across the IPS frequencies. Although the NBR and PBR curves were qualitatively similar, the misalignment between them raised new questions on their coupling.

Previous fMRI studies reported negative BOLD in occipital cortex during visual tasks, but both the stimuli and the properties of NBR were different from our study [8,9]. Stimuli consisted of small flickering target patterns, while here we observed NBR in association with simple IPS. Compared to the mentioned literature, we detected a larger NBR, located in a more peripheral region, the extrastriate visual cortex. Moreover, in our study the NBR regions were plentiful also after multiple comparison correction, thus showing high statistics significance. Previous works made various hypothesis about the origin of NBR. Some authors interpreted the negative BOLD as caused by an hemodynamic effect independent from neural activity, others suggested its coupling to neuronal inhibition. Schmuel et al. suggested that NBR was caused by a local blood stealing phenomenon [8]; this hypothesis was later rejected by Smith et al, who supposed that NBR is caused by a suppression of neural activity [9]. The interpretations are therefore controversial and a full comprehension of the phenomenon is still lacking.

To address the questions on NBR and in order to find further confirmation, we conducted a NIRS study with the IPS protocol on the same subjects. The NIRS and fMRI results were in agreement. Both the GLM analysis led to qualitatively similar activation patterns. Indeed, the NIRS analysis revealed a diffused inverted hemodynamic pattern during the visual stimulation blocks in most subjects. After coregistration of the NIRS optodes to the MR structural images, we estimated the projection of each NIRS channel onto the cortex. The spatial correspondence between the channels and the activated/deactivated regions of one subject is shown in Fig.4. The 3-4 and 4-8 channels, which had the highest statistics significance, were projected exactly over the symmetric NBR regions. This finding strongly confirmed the NBR. In this subject, the fact that no channels had a clear non-inverted response (Fig.3A) was explained by the absence of channels over the PBR area. In this case PBR were restricted to primary visual areas, on the mesial surface of the hemispheres, where NIRS cannot detect signal changes, according to its technical limitations. There were also few channels which remained noisy even after filter correction; their SNR could be improved in future applications by using ad hoc pre-processing steps.

Moreover, the [HHb] and [HbO_2] curves helped to give insight into the NBR characteristics. In previous works on NBR, BOLD recordings were combined with perfusion measurements [8], but, to our knowledge, this was the first attempt to interpret NBR in the light of both NIRS HHb and HbO_2. We found out that the negative BOLD signal was related to an HHb accumulation synchronous with a HbO_2 decrease; the [HHb] variation was highly major in amplitude than the [HbO_2] one.

The demonstration of an inverted hemodynamic pattern is a novel finding also among NIRS studies. Although a group of infants studies reported an increase of [HHb], the finding was often interpreted as ambiguous and related to the choice of NIRS wavelengths [10]. We instead confirmed the inverted hemodynamic pattern by using two independent techniques in combination. The results of both the fMRI and NIRS analyses had a high statistics significance. We therefore attributed the inverted pattern to a physiological phenomenon, resulting from the balance between the oxygen consumption and oxygen delivery mechanisms.

VI. Conclusion

In this study we showed sustained negative BOLD responses to intermittent photic stimulation. We then performed a NIRS study to reproduce these observations. Results provided concordant evidences on NBR. By giving information on both HbO_2 and HHb, NIRS confirmed the NBR and helped to give insight into its physiology.

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