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Group Analysis of fMRI and NIR Data Simultaneously Acquired During Visual Stimulation in Humans

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Abstract

We use our new combined functional near infrared spectro-imaging (fNIRSI) and magnetic resonance imaging (MRI) technique to compare fMRI and fNIRSI data at different activation conditions, to obtain new information about the underlying physiology of the blood oxygen level dependent (BOLD) signal used in fMRI, and to assess statistical characteristics of spatial functional information provided by the group analysis of fNIRSI data. To achieve these goals we have acquired simultaneously fNIRSI and fMRI data during the presentation of the checkerboard reversing with different frequencies, and analyzed these data following the standard correlation and group analysis of variance pathway used in functional neuroimaging. We have found that while the time courses of oxy-, deoxy-, and total- hemoglobin responses are equally well correlated with the time course of the BOLD response, the spatial pattern and magnitude of the BOLD response is better related to those of the oxy-, and total- hemoglobin responses rather than to the deoxyhemoglobin response. The statistical significance of the fNIRSI group maps is inferior to that of fMRI, and can be particularly compromised by the anatomical features of subjects.

Keywords

fMRI; imaging; human brain; photon migration

1. INTRODUCTION

Today the search on the Entrez PubMed engine using the keywords “near infrared” and “human brain” returns about 100 references. However, the majority of these works use near infrared spectroscopy (NIRS,¹) to study human brain without any spatial resolution beyond the location of the optical probe. Adding the word “mapping” to the above list of keywords narrows search results to only about 10 papers. Most of these papers deal with the surface mapping of the functional cerebral hemodynamics², i.e. the locations of the hemodynamic or neuronal changes are projected on the surface of the head with a significant loss of spatial information. Only about five papers^{3–7} employ the three-dimensional functional near-infrared spectroimaging (fNIRSI) to study functional cerebral processes.

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Although general principles of NIRSI have been understood years ago⁸, a particular difficulty in the realization of the functional NIRSI of the human brain is the necessity of information about the shape and internal structure of the head. Very recently we have developed a combined near-infrared (NIR) and functional magnetic resonance imaging (fMRI) system for functional imaging human visual cortex.⁷ In our technique the above problem is resolved by using the structural MRI information. In the present paper we report results of the study of activation in human visual cortex using this combined technique. The goals of the study were to compare fMRI and fNIRSI data at different activation conditions, to obtain new information about the underlying physiology of the blood oxygen level dependent (BOLD) signal used in fMRI⁹, and to assess statistical characteristics of spatial functional information provided by the group analysis of fNIRSI data. In order to achieve these goals we have acquired simultaneously fNIRSI and fMRI data during the presentation of the checkerboard reversing with different frequencies, and analyzed these data following the standard group analysis of variance pathway used in functional neuroimaging.^{10,11}

2. MATERIALS AND METHODS

2.1. NIRSI instrument

The optical signals were recorded using a near infrared spectrometer (Imagent, ISS, Champaign, IL). Data acquisition was synchronized with the fMRI measurements using the TTL-trigger signal from the MR scanner, which also triggered the beginning of the visual stimulation paradigm. The optical sources were laser diodes (690 and 830 nm) which were amplitude modulated at 150 MHz and time-multiplexed. Light reaching the detectors was amplified by photomultiplier tubes and consequently converted into AC, DC, and phase signals for each of the source-detector combinations, or channels, at each wavelength.

2.2. Optical probe

The optical probe was designed with 16 pairs of 400- μ m-diameter core plastic-clad multimode silica source fibers and 4 detector fiber bundles. The physical size of the 3T head-only MR scanner (Siemens Allegra) mandated the use of prisms with dielectric reflective layers for the fiber bundles (metal reflecting surfaces were found to produce significant artifacts in MR images). The optical fibers were “ferruled” using plastic tubing, and the frame of the optical probe was made polyurethane which has appropriate mechanical properties and produces insignificant artifacts in the MR images. The topology of the probe was designed so that the optical channels overlapped and the distribution of source-detector distances covers the optimal range – between approximately 20 and 30 mm, as shown in Fig. 1. An MRI visible marker was attached adjacent to each of the 16 optical source fibers and four detector fiber bundles so that accurate source and detector positions could be estimated from MR images. The thickness of the probe was less than 20 mm to ensure that it could be placed comfortably between the back of the head and the bottom of the MRI birdcage head coil. For the experiment, the center of the optical probe was placed over the primary visual cortex of the subject as close as possible to the calcarine fissure. To ensure the correct location of the probe, we followed a special procedure when installing the probe: before probe installation a vitamin E marker was attached to the subject’s head over the presumed

location of the calcarine fissure, a brief structural MR scan performed and then the optical probe was attached to the best location determined from the obtained MR image.

2.3. MRI setup

For MRI data acquisition we used the 3T head-only MR scanner Allegra (Siemens). The MRI protocol started with a rapid T₁-weighted spin-echo (240×240 mm field-of-view, 512×512 data matrix, 16 slices, 4 mm slice thickness, 0.4 mm inter-slice gap, aligned along the anterior/posterior commissure). These images were used for the initial co-registration with the functional scans, which were acquired using an echo planar imaging (EPI) pulse sequence (slice orientation and dimensions as above, 64×64 data matrix, tip angle 60° , TE 25 ms, TR 2000 ms). After the functional data have been collected, high-resolution ($0.5 \times 0.5 \times 1.0$ mm³) T₁-weighted spin-echo images, termed “probe localizers”, were acquired over a small volume containing the optical probe to enable automatic recovery of the positions of the optodes. Finally, a magnetization-prepared rapid gradient-echo (MPRAGE) 3-D image (spatial resolution $0.94 \times 0.94 \times 1.2$ mm³) of the full-head was acquired for co-registration of the optical and fMRI signals.

2.4. Visual Activation protocol

A pair of non-magnetic goggles (Resonance Technology, Inc.) with LCD screens were placed in front of the subject's eyes inside the birdcage head coil. The block-designed visual stimulation paradigm consisted of five blocks each with 20 s fixation followed by 20 s of a black-and-white checkerboard pattern reversing at certain frequency (1, 2, and 6 Hz). Each block ended with a 20 s relaxation period when subject was presented the black screen. For each frequency of the checkerboard reversing (1, 2, and 6 Hz) a separate five-block experiment was performed.

3. DATA ANALYSIS

3.1. Registration of the optode positions to the structural MR image

Automatic estimation of source- and detector-positions from the MR images, accounting for possible deformations of the flexible optical probe, was performed using custom-developed image processing algorithms implemented in a MATLAB (Mathworks, Natick, NJ) script. The process can be summarized as computing the rotation and translation of the reference coordinate systems of the MPRAGE image with respect to the probe-localizer, determination of the angular position of the cylindrical markers by computing the rotation of the coordinate system due to rotation of the optical probe, and finally correcting for any misalignment from deformation of the probe. The accuracy of the optode localization was comparable with the resolution of the structural MR images (~ 1 mm).

3.2. Optical image reconstruction

Optical images were reconstructed using the AC measurements and the algorithm described in Ref. 7. The key features of the algorithm are the computation of the sensitivity point spread function using Monte Carlo simulations of light transport in the digital head phantom derived from the segmented structural (MPRAGE) MRI image, and the solution of the linearized inverse problem for the local variations of the absorption coefficient using the

SIRT algorithm. The image reconstruction area was restricted to the brain tissue within efficient sensitivity region, which included about 600 cubic voxels with 8 mm sides. Using Lambert-Beer's law^{1,2}, for each voxel of reconstructed 4d (space +time) images the temporal changes in absorption coefficient were converted into changes in the oxy ($[HbO_2]$), deoxy- ($[HHb]$), and total ($[THb] = [HHb] + [HbO_2]$) hemoglobin concentrations.

3.3. Single subject analysis

All 4d images (NIRSI hemoglobin changes and fMRI BOLD) were analyzed in the same way, NIRSI – using MATLAB, and BOLD-using AFNI.¹¹

After the detrending, for each voxel the correlation coefficient between the image value and the 5 second delayed stimulation boxcar function was calculated. Then the images of the mean change between activation and rest conditions were computed and thresholded by the corresponding correlation coefficient value, so that only voxels with correlation coefficient higher than 0.5 were left nonzero. At this stage of the analysis the NIRSI mean change images were converted to the ANALYZE format and then the AFNI software package was used to further analyze both fMRI and NIRSI images. Then all functional images were spatially smoothed with the 4 mm Gaussian kernel, registered to the full head MPRAGE structural images, and transformed to the stereotactic Talairach coordinate system.

3.4. Group analysis

To create group maps for seven remaining subjects (five males and two females), a one-way ANOVA^{10,11} was performed on each voxel in the Talairach space. Mean response images along with corresponding t-statistics images were calculated for each condition (the checkerboard reversing frequency). F-test for equality of the responses across different condition produced the images of treatment effect and of the corresponding F-statistics.¹¹

4. RESULTS

In all axial images presented in this paper left and right sides of the image correspond to the left and right sides of the head, respectively, and the bottom part of the image corresponds to the posterior part of the head. In the figures presenting columns of multiple axial slices (Figs. 4, 5, 6, and 7), slice sequence from top to the bottom of the column correspond to the inferior to superior sequence in the head. Since during functional activation the deoxyhemoglobin concentration usually decreases, the values in the $[HHb]$ maps are inverted so that the hot colors correspond to the negative $[HHb]$, and the cold colors correspond to the positive $[HHb]$. In the $[HbO_2]$ maps hot colors show positive changes, and the cold colors show negative $[HbO_2]$. In the BOLD maps no voxel exhibits negative changes.

Figs. 2(a) and (b) show the single subject BOLD activation maps. The mapped value is the intensity percentage change from 0 to 5 %, and the threshold is set to the correlation coefficient value equal to 0.7. Figs. 1subj (c) and (d) show the corresponding $[HHb]$ unsmoothed maps in the range from 0.2 to 2 μM . The location of the optical probe can be estimated from the markers: one of the source markers is seen on the sagittal image and the

central probe marker is seen on the axial image, so that the probe is well centered to the center of the activated region. A common feature illustrated in Fig. 3(d) is a much larger apparent $[\text{HHb}]$ in the left side of the visual cortex than in the right one. Comparing Figs. 2(b) and (d) one can understand that such an asymmetry in the $[\text{HHb}]$ response is due to the fact that, as one can see in Fig. 1subj (b), in the area of the optical probe location, the superficial cortical layer exhibits stronger activation in the left part of the cortex than in the right one.

The above feature of the BOLD response was found in five subjects and therefore can be also seen in the cross-subject averaged maps of BOLD response (Fig. 3). Figs. 3(a), (b), and (c) correspond to the checkerboard reversing frequencies 1, 2, and 6 Hz, respectively. In Fig. 3(a–c) the color scale is between 0.5 % and 1.8% from green to red. All shown activated voxels are highly significant with p-values less than 10^{-5} . The arrows on the left of the figure show the slices with a prominent left sided protrusion in the response area. Fig. 4(d) shows the map of the treatment effect for the influence of the checkerboard reversing frequency on the BOLD response. The threshold is set to the F-statistics value equal to 4.

Figs. 4 and 5 show the cross-subject mean maps of the $[\text{HHb}]$ and $[\text{HbO}_2]$ response magnitudes, respectively. Panels (a)–(c) in both figures correspond to the checkerboard reversing frequencies 1, 2, and 6 Hz, respectively. In Fig. 4 the hot colors correspond to the range of the negative $[\text{HHb}]$ values from -1.5 to $-5.2 \mu\text{M}$, and the cold colors represent the positive range between 1.5 and $2.0 \mu\text{M}$. In Fig. 6 the hot colors correspond to the range of the positive $[\text{HbO}_2]$ changes from 2.0 to $12.0 \mu\text{M}$ and the cold colors show the negative range between -2.0 and $-6.0 \mu\text{M}$. All optical images (Figs. 5(a–c) and 5 (a–c)) show more prominent hemodynamic changes in the left side of the visual cortex than in the right side in accordance with the feature of the BOLD images discussed above.

The subfigures (d) in Figs. 4 and 5 show the t-statistics maps (averaged over all frequencies) corresponding to the maps in subfigures (a–c). One can see that for both $[\text{HHb}]$ and $[\text{HbO}_2]$ the t-values in the right side of the visual cortex are much smaller than one indicating low statistical significance, while in the left side of the cortex, in the slices corresponding to the region of the best overlap of the single subject maps the t-values are up to 9 for $[\text{HHb}]$ and up to 6 for $[\text{HbO}_2]$.

In the BOLD images, the most prominent change due to the increase in the checkerboard reversing frequency is a noticeably larger number of activated voxels at 2 Hz and 6 Hz compared to 1 Hz (compare panels (b) and (c) with panel (a) in Fig. 3, see also Table 1). The magnitudes of the BOLD response averaged across subjects and over the activated region were close at 1 Hz and 2 Hz, and somewhat higher at 6 Hz (see Table 1).

Both $[\text{HHb}]$ and $[\text{HbO}_2]$ maps show some expansion at 2 and 6 Hz compared to 1 Hz. However, the size of the activated area in the $[\text{HHb}]$ images changes with the frequency in a non-monotonic fashion: the activated area at 2 Hz is larger than at 1 Hz, but at 6 Hz it is smaller than at 2 Hz (compare Figs. 4a, b, and c). The behavior of the $[\text{HbO}_2]$ response maps is more consistent with the BOLD maps: the smallest activated area and magnitude occur at 1 Hz, and the largest occur at 6 Hz (compare Figs. 5a, b, and c). Figs. 6(a–c) show

maps for the changes in the total hemoglobin concentration [THb], whose behavior with the frequency increase is similar to the [Hb02] maps.

All hemoglobin maps in Figs. 4, 5, and 3 contain areas showing increase in [HHb] and decrease in [Hb02] and [THb] during the activation. From Figs. 4(d) and 5 (d) one can see, however, that these areas have low statistical significance. Examination of the single-subject maps revealed that such an “abnormal” behavior of hemoglobin concentrations occurred in the parts of the brain situated far away from the center of the optical probe or deeply in the brain. The most plausible explanation of such a behavior is that it is the artifact resulting from the poor performance of the SIRT algorithm in those areas.⁷

In Fig. 4(d), 5 (d), and 6 (d) one can see that the area showing the strongest frequency effect on the BOLD response is within the area having highest t-values both for [HHb] and [Hb02] maps. The values for [HHb], [Hb02], and [THb] shown in Table 1 were calculated by averaging over the regions in the corresponding maps having t-values higher than 2. In Table 1 one can see that the magnitude of [HHb] remains approximately the same at all frequencies, while the magnitudes of [Hb02] and [THb] exhibit slight elevations with the frequency increase.

Although the changes in [HHb], [Hb02], and [THb] shown in Table 1 are expressed in μM , we do not discuss their absolute values because, as it is commonly believed⁴, the uncertainty in the background optical properties of the head tissues results in the biasing of the absolute hemodynamic response measures.

5. DISCUSSION

Our results show that the cross-subject mean functional maps of BOLD, [HHb], [Hb02], and [THb] responses to visual stimulation undergo changes when the frequency of the checkerboard reversing increases. The dependence of the BOLD response on the visual stimulus frequency is in a fair qualitative agreement with that reported in Ref. 12. The new result provided by fNIRSI is that spatial behavior of the [Hb02] and [THb] responses are more consistent with the BOLD response than the spatial behavior of the [HHb] response. Changes in the mean ROI magnitude of [Hb02] and [THb] also appear to be better linked to the changes in the BOLD signal magnitude than those for [HHb].

In our previous NIR study of the BOLD response in the motor cortex we found that the time course of the BOLD response is much better correlated with the time course of the – [HHb] signal than with that of the [Hb02] and [THb] responses. However, in the present study, we find that in the visual cortex the – [HHb] and [Hb02] time courses have statistically equal correlation coefficients with the BOLD signal. The [THb] signal is slightly less correlated with the BOLD one, however, given that [THb] is simply the sum of [HHb] and [Hb02], and that the standard deviation of the mean correlation coefficient between [THb] and BOLD is close to the difference between this value and that for [Hb02] and – [HHb], one can conclude that this difference is due to the higher noise in the [THb] rather than due to the stronger time course mismatch. This result is in agreement with our another previous study¹³ in which we found a qualitative difference in the relative time course of – [HHb] and [Hb02] in the motor and visual cortices: in the visual cortex the

patterns of $-[HHb]$ and $[HbO_2]$ were strongly linearly correlated, while in the motor cortex $[HbO_2]$ reached its maximum a few seconds before $[HHb]$ reached its minimum.

Our new findings do not contradict the fact that the temporal changes in the BOLD contrast follow the time course of the $-[HHb]$ signal. However, they indicate that the spatial distribution of the BOLD response and its magnitude are more related to the corresponding characteristics of the $[HbO_2]$ and $[THb]$ responses. These two features can be combined if one assumes that in the visual cortex the magnitude of the BOLD signal is determined by the magnitude of changes in the total hemoglobin concentration $[THb]$. This scenario is different from the motor cortex one, but agrees with the general model of the BOLD signal proposed in Ref. 9. This finding indicates that BOLD effect has complex physiological nature and simultaneous measurements using fNIRSI are important to fully understand the underlying physiology of functional activation in the brain.

Although fNIRSI functional data demonstrate certain reasonable correlation with fMRI, from the statistical comparison of NIRSI images and fMRI it is obvious that the group mean functional NIRSI images have much lower statistical significance than the fMRI those. Three main reasons for that are a lower signal-to-noise ratio of NIRSI, its image reconstruction artifacts, and the mismatch in the optical probe locations among different subjects. While there is a constant ongoing research on the image reconstruction improvement, including that in our group, two other problems can be resolved by repeating measurements on the same subject multiple times outside of the MRI scanner. When doing this, researchers should take into account the fact that, as in our study, one side of the visual cortex can produce better functional NIR signals than another.

Kaufmann et al. reported larger amplitude of the BOLD response in the right hemisphere. We have not observed any lateralization in the BOLD response magnitude, but rather we observed more superficial response in the left hemisphere. The reason for this can be purely anatomical, namely that in the area of the primary visual cortex the left hemisphere is more protruded than the right one. We observed such a feature in four out of seven subjects whose data were included into the analysis.

6. CONCLUSION

We have performed a group statistical analysis of simultaneously acquired fNIRSI and fMRI data on the human primary visual cortex during different visual activation conditions. We have found that while the time courses of oxy-, deoxy-, and total- hemoglobin responses are equally well correlated with the time course of the BOLD response, the spatial pattern and magnitude of the BOLD response is better related to those of the oxy-, and total- hemoglobin responses rather than to the deoxyhemoglobin response. The statistical significance of the fNIRSI group maps is inferior to that of fMRI, and can be particularly compromised by the anatomical features of subjects. This drawback can be reduced by additional measurements by fNIRSI and further technology development.

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Fig. 1. Schematic of the optical probe. The probe has 16 pairs of source fibers and 4 detector fiber bundles.

Fig. 2.
(a) and (b): Single subject BOLD activation maps, (c) and (d): Single subject [HHb] response maps.

Fig. 3.

(a)–(c): Cross-subject averaged maps of the BOLD response at checkerboard reversing frequencies 1, 2 and 6 Hz, respectively; (d): map of the treatment effect for the influence of the checkerboard reversing frequency on the BOLD response. The threshold is set to the F-statistics value equal to 4.

Fig. 4.

(a)–(c): Maps of the cross-subject mean [HHb] response magnitude at checkerboard reversing frequencies 1, 2 and 6 Hz, respectively, (d): a t-statistic map for the [HHb] response at 1 Hz.

Fig. 5.
(a)–(c): Maps of the cross-subject mean [Hb02] response magnitude at checkerboard reversing frequencies 1, 2 and 6 Hz, respectively, (d): a t-statistic map for the [Hb02] response at 1 Hz.

Fig. 6.
(a)–(c): Maps of the cross-subject mean [THb] response magnitude at checkerboard reversing frequencies 1, 2 and 6 Hz, respectively.

Table 1

Statistical data

Frequency	Number of BOLD activated voxels	BOLD (%)	[Hb02] (μM)	– [HHb] (μM)	[THb] (μM)
1 Hz	14880	1.06 ± 0.11	3.3 ± 1.3	2.1 ± 0.5	1.2 ± 1.4
2 Hz	22701	1.08 ± 0.11	4.1 ± 0.9	2.0 ± 0.7	2.1 ± 1.2
6 Hz	26466	1.14 ± 0.11	4.9 ± 0.8	2.1 ± 0.5	2.8 ± 0.9
Correlation coefficient with BOLD averaged over all subjects and frequencies			0.82 ± 0.09	0.82 ± 0.05	0.71 ± 0.1