Combination of event-related potentials and functional magnetic resonance imaging during single-letter reading

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Abstract—This work proposes a mathematical approach for combining event-related potentials (ERPs) and functional magnetic resonance images (fMRI). Data were separately recorded during the same event-related experimental design, consisting of visually presented single letters and non-alphabetic symbols, that had to be either simply observed (*passive* condition) or read aloud (*active* condition). This protocol was useful for exploring the neural correlates of reading processes. Healthy adults participated in the experiment. Averaged ERPs were decomposed by Independent Component Analysis; Low Resolution Electromagnetic Tomography (LORETA) was applied to estimate the current density distribution maps of each independent component. fMRI images time series were analyzed by multiple linear regression. ERP-fMRI correspondence was quantified by computing the Euclidean distance between LORETA local maxima and clusters of significantly activated fMRI voxels. During reading aloud of letters, that is clearly the task most similar to natural reading conditions, significant electrical and hemodynamic response was observed in the left medial frontal gyrus (BA 6) and left middle temporal gyrus (BA 22/39) just before articulation and in the bilateral middle superior temporal gyrus (BA 22/37) during and after verbal-motor production. These results indicate that the middle-superior temporal gyrus plays a crucial and multifunctional role in grapheme-phoneme matching.

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I. INTRODUCTION

ELECTRICAL and hemodynamic measurements of brain activity are functionally coupled in time and space [1]: specifically, neural synaptic activity generates an increase of metabolic load and thus of oxygen consumption, that in turn causes an over-compensatory increase of blood flow and oxygen delivery.

Event-related potentials (ERPs) are capable of measuring electrical brain activity in real-time, but do not allow precise identification of neural generators due to non-uniqueness of solution of the "inverse problem". On the other hand, functional magnetic resonance imaging (fMRI) indirectly quantifies local changes of blood oxygenation with mm precision, but temporal resolution is greatly limited by the sluggishness of the hemodynamic response function.

For these reasons, individually applied ERPs and fMRI do not provide measurements of brain activity with satisfactory temporal and spatial resolution. Therefore, a multimodal approach might be necessary for correctly investigating the spatiotemporal dynamics of complex brain functions. This need particularly applies to complex cognitive functions that involve sensory, motor and cognitive systems, each consisting of a spatially distributed neuronal assembly responsible of a temporally well-localized stage of information processing. For example, reading functions engage a broad network of brain regions, including the left inferior parietal cortex (BA 40), precuneus (BA 7) and inferior frontal gyrus (BA 6/44), the bilateral fusiform gyrus (BA 19/37) and supplementary motor area (BA 6) [2], [3]. The investigation of the time course of these areas [4]-[6] has shown that the visual characteristics of letters/words are analyzed between 50 and 100 ms after stimulus presentation, thus generating early perceptive potentials. The conversion from visual to linguistic analysis likely occurs between 150 and 230; the brain regions responsible for higher order cognitive functions, such as semantic analysis, feedback processes and memory are supposed to be recruited at longer latencies.

Here we propose a multimodal approach that combines separately recorded ERPs and fMRI data in order to exploit their complementary spatiotemporal resolution. This procedure is interestingly applied to the temporal dynamics of the several brain regions engaged by single-letter reading.

As compared to previous ERP-fMRI study of reading functions [7], our multimodal approach is expected to be helpful for highlighting the neurobiological and functional basis of reading in both healthy and impaired readers, since the identification of letters, other than words, has been shown to predict reading success and to distinguish adult dyslexics [3]. Moreover, we applied advanced methods to decompose ERPs into independent waveforms, likely related to functionally different cerebral sources.

II. METHODOLOGY

A. ERP Analysis

ERP morphology is usually described in terms of latency and amplitude of the main peaks and troughs; however, generally there is not a one-to-one correspondence between single deflections and discrete stages of information processing. In the present work, Independent Component Analysis (ICA) was applied to estimate spatially fixed and temporally independent components (ICs), that better represented distinct functional processes in comparison with single ERP deflections. This method extracts n components from a set of n signals: however, since the number of neural sources is actually lower than the number of recording electrodes, Principal Component Analysis (PCA) was applied before ICA to reduce data dimensionality. Averaged ERPs were reconstructed from the first m principal components (PCs) accounting for more than 95% of data spatial variance; then, the *runica* algorithm as implemented in EEGLAB toolbox (http://sccn.ucsd.edu/eeglab) [8] was applied, thus obtaining m ICs. The time course and scalp topography of ICs were inspected for defining a correspondence with the physiological potentials traditionally identified on ERPs.

Spatial distribution of neural generators was estimated by Low Resolution Electromagnetic Tomography analysis (LORETA) as implemented in LORETA-KEY$^{©®}$ software (http://www.unizh.ch/keyinst/NewLORETA/LORETA01.htm) [9]. This method finds the smoothest linear 3D solution to the inverse problem, without *a priori* assumptions about the number of sources: solution space consists of 2394 7 mm^3 voxels, belonging to gray matter and hippocampus registered to Talairach atlas [10]. LORETA was applied to single ICs: current density distribution maps were transformed to z-score and then thresholded at $z = 1.96$, corresponding to a two-tailed probability of $P < 0.05$. Source configuration was described in terms of supra-threshold LORETA local maxima.

B. fMRI Analysis

Functional images were analyzed using Analysis of Functional Neuroimages (AFNI) software [11]. Pre-processing steps included slice timing correction, rigid head movements correction, spatial smoothing with a 5-mm full-width high-maximum (FWHM) Gaussian filter and signal intensity normalization. Statistical analysis of functional images time series was performed with multiple linear regression: the impulse hemodynamic response function (HRF) was modeled as a linear combination of 3 basis functions (Fig. 1). Additional regressors were included in the model as confounds: baseline quadratic drift, head rotation and translation parameters. General linear tests were performed to identify active regions for each specific task. Omnibus F-test thresholded at $P < 10^{-3}$ was used as a mask: the null hypothesis $\beta_1+\beta_2+\beta_3 \neq 0$ was tested for each task separately with a t-test analysis and thresholded at $P < 10^{-2}$.
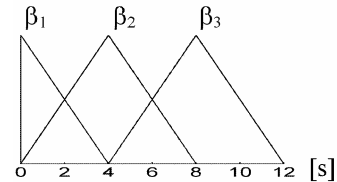

Fig. 1. Triangular basis functions used for modeling the temporal profile of the impulse HRF. β_1, β_2 and β_3 are the regression coefficients.

Group analysis was performed by 2-way ANOVA with mixed effect model.

C. ERP-fMRI Combination

Combination of current density distribution maps and functional activation maps was obtained by computing the Euclidean distance between LORETA local maxima and clusters of fMRI activated voxels, for each IC and task. We considered that a region had significant electrical and hemodynamic activity at the same time if the Euclidean distance was shorter than 15 mm: this tolerance perfectly matches the low spatial resolution of LORETA maps (7 mm^3) and the results of previous studies [7], [12].

III. EXPERIMENTAL PROTOCOL

Eight healthy adults participated in the experiment (age 22-36 years). ERPs and fMRI were separately recorded during the same event-related experimental design.

Stimuli consisted in single letters and non-alphabetic symbols visually presented for 20 ms. Inter-stimulus interval (ISI) was randomized and was multiple of 500 ms; minimum ISI was 2 s during fMRI scanning and 4 s during ERP recording. *Passive* condition consisted in simply watching at randomly ordered letters and symbols (*letter presentation* – LP and *symbol presentation* – SP tasks); *active* condition consisted in reading aloud the letters (*letter recognition* – LR task). There were 150 events for each task type. Stimulus timing was decided considering that the HRF of each task could be estimated with the least amount of unexplained variance.

EEG was recorded from 19 electrodes referred to right mastoid, integrated in an elastic cap and placed according to the standard 10-20 system. For further analysis, EEG was low-pass filtered and sub-sampled at 128 Hz. During the

test, subjects were sitting in a dimly illuminated, electrically and acoustically shielded room and stimuli were displayed on a CRT screen.

Multi-slice echo-planar images (EPIs) were axially acquired on a 1.5 T MRI scanner (GE Medical Systems Signa) with TE = 40 ms, TR = 2 s, FOV = 240 mm with 64 x 64 acquisition matrix and 22 contiguous 5-mm slices (3.75 mm × 3.75 mm in-plane resolution). High resolution structural scan was acquired in the sagittal plane using a 3D GRASS sequence with TE = 5.22 ms, TR = 12.1 ms, FOV = 240 mm with 256 x 256 acquisition matrix and 120 slices (0.94 x 0.94 x 1.2 mm voxel size). Subjects' head was carefully blocked by external means; stimuli were projected onto a screen located near the bottom of the bore and viewed from a mirror mounted on the head coil.

IV. RESULTS

For all tasks and subjects, two ICs were respectively associated to N2_P2b potential (a negative peak at about 180 ms followed by a positive peak at about 300 ms, with posterior distribution) and to P2a potential (a positive peak at about 200 ms with frontal-central distribution). During LP and LR, a further component was associated to a wide and slow negativity occurring between 0.5-1 s with frontal (LNAf) and posterior distribution (LNAo) respectively.

LORETA maps showed that the medial frontal areas and the middle temporal lobe were early engaged by N2_P2b potential, on the left during reading aloud and bilaterally during passive tasks. Next, the middle-superior temporal gyrus bilaterally (BA 22/39) was strongly activated in correspondence to P2a during all tasks. At long latencies, despite a different topographic distribution, LNAf/LNAo components had partially common sources in the middle-superior temporal gyrus bilaterally (BA 21/22/37/39/42).

According to fMRI results, the left inferior parietal lobule (BA 7/40) and medial frontal gyrus (BA 6) were commonly activated during LP and LR, while bilateral pre- and post-central gyri (BA 3/4), left middle frontal gyrus (BA 46) and left superior temporal gyrus (BA 22/47) were additionally engaged during reading aloud. Passive tasks were characterized by activation of the left fusiform gyrus (BA 19/37) and of the pre-central gyrus (BA 4/6) (left hemisphere during symbol presentation and right hemisphere during letter presentation).

Brain regions with significant electrical and hemodynamic activity are summarized in Table I. Significant ERPs-fMRI correspondence was observed during LP in the left middle temporal gyrus (BA 39) for all the independent components; during SP in the left middle temporal-occipital gyrus (BA 19/39) for N2_P2b component; during LR in the left medial frontal gyrus (BA 6) and left middle temporal gyrus (BA 22/39) for N2_P2b component and in the middle-superior temporal gyrus bilaterally (BA 22/37) for P2a and LNAo components (Fig. 2).

V. DISCUSSION

Similarly to previous researches [7], we propose to combine the results of data analysis of separately recorded ERPs and fMRI, without constraining *a priori* the configuration of the sources.

From a psychophysiological perspective, our approach might be defined as "ecological", since single-letter reading allows to evaluate the simplest unit of grapheme-phoneme association mechanisms in the most natural conditions. Furthermore, the same event-related design applied to brain potentials was exactly reproduced in the fMRI session: this is a fundamental pre-requisite for properly combining the two methodologies, requires advanced statistical analysis of fMRI data, and usefully enhances the temporal differences between BOLD and speech-related artifacts. Methodologically, we combined traditional and innovative

TABLE I
REGIONS WITH SIGNIFICANT ELECTRICAL AND HEMODYNAMIC ACTIVITY BY TASK AND INDEPENDENT COMPONENT

		N2_P2b					P2a					LNAf/o				
		Name	x	y	z	E	Name	x	y	z	E	Name	x	y	z	E
LP	L	Middle Temporal (BA 39)	-51	-58	10	12	L Middle Temporal (BA 39)	-51	-58	10	14	L Middle Temporal (BA 39)	-58	-38	3	12
SP	L	Middle Temporal-Occipital (BA 19/39)	-51	-38	3	13										
LR	L	Middle Temporal (BA 22/39)	-58	-37	9	12	L Middle-Superior Temporal (BA 22/37)	-58	-38	3	8	L Middle-Superior Temporal (BA 22/37)	-58	-37	9	10
							R Middle-Superior Temporal (BA 22/37)	52	-52	-8	15	R Middle-Superior Temporal (BA 22/37)	52	-58	-2	15
	L	Medial Frontal (BA 6)	-3	-8	46	15										

L = left, R = right hemisphere; BA = Brodmann's Area; x = sagittal, y = coronal; z = axial coordinates in Talairach and Tournoux (1988) stereotaxic atlas [4] (minus signs indicate left hemisphere, posterior to anterior commissure and inferior to the anterior-posterior commissures line, respectively); E = Euclidean distance between LORETA local maxima and fMRI activated clusters.
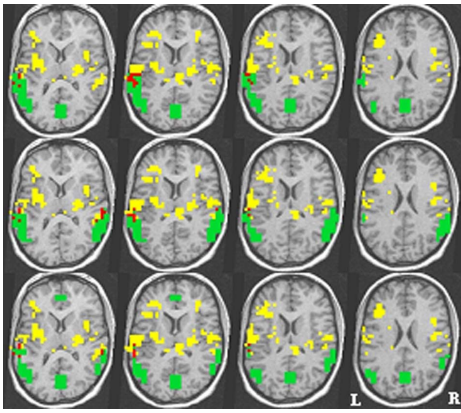
Fig. 2. LR task: superimposition of fMRI activation maps (yellow) and LORETA maps (green) of N2_P2b (top row), P2a (middle row) and LNAo (bottom row): red regions indicate overlap (from z = +9 mm to z = +21 mm, gap 4mm). L = left; R = right

analysis strategies of ERP data by defining the correspondence between ERP peaks and independent components estimated with ICA: this method allows to focus the subsequent source analysis on spatially fixed and temporally independent components likely representing functionally distinct stages of information processing.

N2 potential may be related to reafferent activity following articulation, since its amplitude reduction in pre-central compared to posterior regions may represent an inhibitory effect due to voluntary verbal-motor production [13]. P2a and P2b potentials have different latencies and scalp topographies and are represented in two different ICs: this observation further support the hypothesis that they are likely generated by different brain sources. Magneto-encephalographic studies [4]-[6] have suggested that P2a is related to preparation to vocalization and to stimulus categorization, while P2b represents an interface between visual and linguistic domains.

Estimated neural sources indicate that attentional processes mainly located in the medial frontal gyrus are selectively activated by letter reading just before articulation. Furthermore, bilateral engagement of the middle-superior temporal gyrus in correspondence to P2a potential during all tasks suggests that this region is crucial for stimulus categorization. The physiological meaning of LNAf/o is not precisely known and their marked variability makes difficult to reliably identify these potentials on individual ERPs. However, their absence during symbol presentation suggests that they may be specific of phonological processing.

Considering fMRI results, the letter-specific activation observed in the left inferior parietal cortex (BA 7/40) and medial frontal gyrus (BA 6) is consistent with results obtained by [2], [7]. Engagement of the fusiform gyrus bilaterally (BA 19/37) had already been related to attention to isolated letters and symbols [3]: in the present study, activation was left-lateralized. Articulatory movements clearly recruit the pre- and post-central gyri bilaterally (BA 3/4) during reading aloud of letters.

LORETA and fMRI maps are partly similar, but also show some differences that can be attributed to the still poorly known neurovascular coupling mechanism, and to the different signals that the two methodologies are able to detect, i.e. ERPs measure the output while fMRI the input of the neuronal activity [1]. For all tasks, the cortical region with the widest inter-modality similarities is the middle-superior temporal lobe (BA 19/22/37/39). In the letter presentation task similarity is left lateralized, while during the letter recognition task left predominance is limited to N2-P2b component and becomes bilateral for later components (P2a and LNAo). During letter recognition, the left medial frontal gyrus (BA 6) additionally shows similar electrical and hemodynamic activity. The middle-superior temporal gyrus receives inputs from the visual system and strongly interacts with temporal auditory areas: therefore, its role in linguistic and reading processes is crucial and multifunctional. The spatial location and the high interconnectivity with the main sensory systems may have favored the specialization of this region for phoneme-grapheme matching.

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