SIMULTANEOUS RECORDING OF VEP AND fMRI TO STUDY OPTIC NEURITIS IN MULTIPLE SCLEROSIS

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ABSTRACT
Multiple sclerosis (MS) is a chronic, inflammatory disease that affects the central nervous system and causes gradual destruction of myelin. Inflammation of the optic nerve (optic neuritis, ON) is a common symptom at the onset of MS. Visual evoked potentials (VEPs) have been widely used as diagnostic tools for ON as they can detect the malfunctioning of visual pathways. Functional Magnetic Resonance Imaging (fMRI) has also been used to identify impaired visual brain function in ON patients. As VEPs and fMRI have complementary features (high temporal resolution and high spatial resolution, respectively), the integration of simultaneously recorded VEP and fMRI might provide more detailed information on the cortical representation of the visual stimulus, thus improving the diagnosis of ON.

In this work, we present a method for simultaneous acquisition of EEG signals and fMRI. More specifically, we show that reliable VEPs can be obtained during fMRI acquisition and, conversely, sequences of MR images of good quality can be obtained in presence of EEG electrodes in the MRI bore. On the basis of these preliminary results, we suggest that the simultaneous recording of VEP and fMRI is feasible and can be used in studying ON.

KEY WORDS
Visual Evoked Potentials (VEPs), Functional Magnetic Resonance Imaging (fMRI), Electroencephalography (EEG), Optic Neuritis (ON)

1. Introduction
Inflammation of the optic nerve (optic neuritis, ON) manifests itself as an acute loss of vision, associated with pain. ON is a common symptom at onset of multiple sclerosis (MS). ON can be studied by means of Visual Evoked Potentials (VEPs) and functional Magnetic Resonance Imaging (fMRI). VEPs allow observing the brain activity in response to visual stimuli at a millisecond time scale [1]. fMRI exploits changes in the blood oxygenation level dependent (BOLD) signal due to neuronal activity in order to localize brain activation with a high spatial resolution [2]. Alteration of VEPs during pattern stimulation is considered as one of the most characteristic EEG signs in MS, being present almost invariably in subjects affected by ON [3]. Therefore, VEPs and motor evoked potentials have been widely used for the assessment of the course of multiple sclerosis [4]. In ON patients, the latency of the major positive component (P100) of VEP in the affected eye is significantly increased as compared to the unaffected eye and in normal volunteers [5]. A few fMRI studies, to date, have investigated the effects of ON. More specifically, the size and extent of the BOLD response induced by monocular checkerboard stimulation in the ON group was reduced in affected eyes compared to unaffected eyes. Therefore, fMRI might be useful in following the evolution of the resulting functional deficit in individual subjects [6]. Some studies [7] apply both methods separately and integrate the information provided by the two measures. As VEPs and fMRI have complementary features (high temporal resolution and high spatial resolution, respectively), their integration might provide even more detailed information than either method alone [8]. As evoked cortical responses inherent variability (and of their hemodynamic correlates measured by fMRI), the integration of both methods would be more reliable if electrical potentials and hemodynamic responses were recorded simultaneously. The simultaneous measurements ensure that fMRI and electroencephalographic (EEG) recordings reflect exactly the same states of brain activity [9, 10]. In optic neuritis, simultaneous recording of VEPs and fMRI data might provide precise informations on the processing of visual stimuli. Up to our best knowledge, no study addressed the simultaneous acquisition of EEG and fMRI data in ON patients.

Here we present a method for simultaneous acquisition of VEPs and fMRI, and show its application to MS subjects with ON symptoms.
For each electrode, the electrode-skin impedance was maintained less than 5 kΩ. The head was fixed in the head coil, and electrode leads was immobilized with tape to avoid movement-related artefacts. The cap was connected to the amplifier with a dedicated a-magnetic cable immobilized with tape, laying on the ground and following a straight path. An electrocardiogram (ECG) and the trigger signal were also recorded along with 19 monopolar EEG channels. The electrophysiological recordings were performed at a 4 kHz sampling rate, with a dynamic range of 128 mVPP (20-bit resolution). The acquired EEG data were stored for offline processing.

2.3 Anatomical and functional MRI data acquisition

MR images were acquired on a 1.5 T scanner (General Electric, Milwaukee, USA), at the Magnetic Resonance Research Centre on Nervous System Diseases of the University of Genoa. A phase-array coil was used, in order to have good signal in the occipital cortex, which is the object of this study.

High-resolution anatomical images were acquired using a 3D SPGR (Spoiled Gradient Recalled) sequence (TR=30 ms; TE=3 ms; slice thickness=3 mm; FOV=240 mm; Matrix: 256×256; Flip Angle=35°). Functional images were acquired using echo-planar imaging (EPI) sequence to obtain 20 contiguous slices for each brain volume (TR=3000 ms; delay=1000 ms; TE=50 ms; FOV=260 mm; Matrix=64×64; slice thickness=6 mm). During the acquisition of Echo Planar Imaging (EPI) sequences, the stimulation protocol was run according to a block design, as explained in the Figure 1. Grey phases were used as rest condition in fMRI analysis. A T2-weighted volume was obtained with the same slices as the functional scans (TR=6000 ms; TE=122.90 ms; slice thickness=6 mm; FOV=240 mm; Matrix: 512×512; Flip Angle=90°).

For the MS patient, a clinical imaging protocol was also used for a morphological study of the optic nerve, including a STIR IR (Short T1 Inversion Recovery) sequence (TR=2000 ms; TE=40 ms; slice thickness=3 mm; FOV=220 mm; Matrix: 128×256).

2.4 Analysis of fMRI sequences

fMRI volumes were analyzed with SPM2 (Wellcome Department of Cognitive Neurology, London, United Kingdom; http://www.fil.ion.ucl.ac.uk/spm) implemented in Matlab 6 (Mathworks, Inc., Sherborn MA, USA) as described elsewhere [11, 12]. All the images were realigned to the first image acquired for each stimulation condition, and a mean functional image was created. The mean functional images were then normalized to the Montreal Neurological Institute brain template, and the resulting transformation matrix was applied to the individual functional volumes. Finally, the images were smoothed with a 12 mm full-width at half maximum (FWHM) Gaussian kernel. Statistical analyses were performed using the general linear model [13]. Statistical t-test contrasts were conducted, at 2 rev/sec 1 and 4 c/d; 8 rev/sec 1 and 4 c/d, with respect to the resting state condition (i.e. the presentation of uniform grey), and the corresponding activation maps were calculated.
Activations surviving an uncorrected threshold of \( P < 0.001 \) were considered as statistically significant. Tables with activated clusters and the corresponding Brodmann Areas (BA) were also obtained (not shown here).

### 2.5 Artifact removal in EEG recordings

fMRI artifacts were removed from each data individually by the EEG acquisition and filtering software. The filtering algorithm calibrates itself from the first useful MR sequences. When the EPI sequence (dynamic) runs, the filter enters in calibration mode, and only few scans are needed to build a robust dynamic model, where all the events related to each volume acquisition (i.e. number of slices/volume, TR, TE, RF pulses, Gradient selection and Readout Gradients) are measured and averaged to compute the best settings. This method was validated in [14], which shows that the morphology and the amplitude of the spike activity during the MR (EPI) sequence was not changed with respect to that recorded beforehand and afterwards. We used the same algorithm for filtering the EEG signal to obtain visual evoked potentials that were free from MR artifacts (Figure 2). To ensure that the information in VEPs has been preserved after filtering MR artifacts in a simultaneous recording, we trained the model of the filter for the optimal parameters with EPIs without visual stimulation, i.e. first 20 seconds grey. In this way, we ensured that any response elicited by the checkerboard would not be filtered out.

### 2.6 VEP estimation

After gradient artifact reduction with time-based adaptive algorithm, the data were down-sampled at 512 Hz and filtered with a 0.3-30 Hz band-pass filter for further analysis. Eye blinking and eye movement artifacts were removed by visual inspection. We then estimated visual-related potentials at each electrode.

To characterize the response to low-frequency stimulation (i.e., 2 rev/sec), we computed transient VEPs (T-VEP) by averaging the event-related potentials (120 stimuli for each eye). We then estimated the latencies of the P100 component of the T-VEP response.

For high-frequency stimulation (8 rev/sec), we calculated steady-state VEPs (SS-VEP) responses by estimating the transfer function between the trigger stimulus and the EEG signal (480 stimuli for each eye). We then estimated the coherence level and the phase lag at the second harmonic of the stimulus (8 Hz). In the Results, the phase lag is converted to time lag for that frequency.

### 3. Results

#### 3.1 fMRI

In the case of the patient reported here, the affected eye was the left one; in the STIR image an area of T2 hyperintensity can be noticed at the level of the left optic nerve (Figure 3).
The signal to noise ratio (SNR) was estimated in order to evaluate possible disturbances induced in imaging by the EEG acquisition system. The SNR was calculated as the ratio between the mean intensity value of a small region of interest (ROI) placed in the white matter inside the thalamus, which is a very homogeneous region of tissue with high signal intensity, and the standard deviation of a large ROI placed outside the brain in the background, by avoiding ghosting or other artefact regions (Figure 4a). The SNR in EPI images acquired during EEG recording was compared with the SNR of EPI images in absence of EEG electrodes. We calculated the SNR for different volumes within the course of each experiment and compared the corresponding volumes (i.e. same experimental phase) in five different experiments. Data were spread (data from one volume are represented in Figure 4b); no significant decrease in SNR was evident in case of EEG recording. The stimulation at 8 rev/sec does not allow discriminating the single responses of electrophysiological signals, but it produces the maximum of hemodynamic response in the fMRI studies (Figure 5).

3.2 T-VEP
We observed a greater latency (about 140 ms) in the P100 component of T-VEP by stimulating the affected eye of the MS patient at low-frequency (2 rev/sec), while this finding was absent in the normal eye (Figure 6a).

3.3 SS-VEP
At high-frequency stimulation (8 rev/sec), the response of the affected eye at 4 c/d revealed smaller amplitude than that of the non-affected eye (Figure 6b, c). The latency increases from the normal eye to the affected eye of the patient while remains almost constant in between two eyes of control subject (Figure 7a). We used coherence as an indicator for the magnitude of the stimulus-evoked response on a particular brain area, particularly, at O1 and O2 (Figure 7b). As expected, the coherence between stimulus and affected eye (A) of the patient is very low as compared to the same eye of a normal subject (N), which indicates the absence of response in the affected eye. Phase distribution (converted in ms) was plotted on head plots (Figure 7c): the affected eye showed a larger lag to the stimulation whereas the normal eye has low phase lag at occipital sites.

3.4 Integration of VEP and fMRI
We combined the results of VEP and fMRI at 8 rev/sec in (Figure 8). The response of the normal and the affected eye of the patient was plotted as latency and coherence of VEP against number of activated volume in fMRI. The affected eye (A) has a longer latency of P100 than that of the normal eye (N); the activated volume in visual areas (BA 17,18 and 19) of the patient is less than that of the control.
4. Discussion

EEG measurements recorded during fMRI reveal a number of problems like cardio-ballistic pulse artifact (PA) and much stronger gradient related artifacts[9, 10]. A disadvantage of simultaneous recording approaches is the loss of a portion of the EEG information, which might be relevant for the specific issue. This has been addressed in [15], where the VEPs was reconstructed reliably from periods during MR scanning and in between successive scans. We tried to answer the possible questions regarding the accuracy of our method. First, do VEPs (T-VEP and SS-VEP) change during simultaneous recordings? Second, how the results are successful in remarking the periods during MR scanning and in between successive scans. We tried to answer the possible questions regarding the accuracy of our method. First, do VEPs (T-VEP and SS-VEP) change during simultaneous recordings? Second, how the results are successful in remarking the periods during MR scanning and in between successive scans. An evoked potential is elicited to each stimulus, and averaged response is time locked to the stimulus and can be measured by amplitude and latency. P100 component is a positive component that in young healthy subjects shows a peak at about 100 ms after the onset of visual stimuli and represents the most important cortical evoked responses to them. We found a different response to stimulation at 2 rev/sec-1 c/d between both eyes of the ON patient. An increase in the latency is consistent with the previous findings of ON [16]. Steady state (SS-VEP) responses are elicited by high repetitive frequency (in our case 8 rev/sec); the second harmonic response is a major component of the pattern reversal steady state stimulation due to nonlinearities in the visual system. At 1 c/d the both eyes shows the same behavior but a sharp decrease in VEP amplitude is observed at high spatial frequencies in the affected eye while this affect was low in normal eye (Figure 6 b,c). We calculated coherence as an indicator of evoked response on particular brain areas, particularly, at O1 and O2. The coherence for the affected eye is nearly zero at high temporal frequency (Figure 7b). It indicates that the second harmonic response in the affected eye is absent while the occipital sites of the normal eye does not show this behavior. For high-frequency stimulation, (Figure 7a) shows data from one control and one patient, the affected eye has a longer delay in response to the stimulus than normal eye while the delay is almost constant in the two eyes of control subject.

5. Conclusion

In patients affected by MS, ON is a common cause of visual loss [17]. For a long time VEPs have been used for the diagnosis and follow-up of ON[18], and a recent work confirmed the importance of VEPs in the management of ON[19]. On the other hand, it could be very useful to have precise and accurate data for the evaluation of the damage caused by the pathophysiological process of MS in the visual pathways. Despite the difficulty of investigating the optic nerve, the new developments in
neuroimaging are highly promising, considering also the presence of artefacts of EPI sequences [20]. Aim of this study was to explore the functionality of the optic nerve in MS by exploiting both the spatial resolution of fMRI and the temporal resolution of VEPs. As we have explained in the Results, when the affected eye of the patient was stimulated, data showed an increase in the latency of the P100 component of T-VEP and an increase in the phase-lag in SS-VEP, while we obtained a bilateral activation in visual cortex (Brodmann Areas 17, 18, 19) both when stimuli were presented to the affected eye of the patient or to the normal eyes of the patient and of the control, but the activation area in the patient was reduced than that of the control. Preliminary results can be explained by the pathophysiological characteristics of MS: in the acute phase of ON, the inflammation causes destruction of the myelin sheaths (a process called demyelination) rather than an evident neuronal loss. Because normal conduction of the nerve impulse depends on the insulating properties of the myelin sheath surrounding the axon, the demyelinated regions of the optic nerve do not conduct an impulse as well as the not affected regions. So, ON can lead to alterations in VEPs like that we found by stimulating the affected eye of the patient [22]. In fMRI, in ON, the volume of activation in the primary visual cortex is dramatically reduced in response to stimulation of the affected eye [6]. On the basis of our preliminary results we suggest that the simultaneous recording of VEP and fMRI is feasible and can be used in studying ON.

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References