Dynamical Cortical Activations Associated with Saccade Execution: A Normalized Integrative fMRI-MEG Study

Hiroaki Natsukawa, **, # Tetsuo Kobayashi*

Abstract To validate the performance of a newly developed normalized integrative functional magnetic resonance imaging-magnetoencephalography (fMRI-MEG) method and identify the temporal and spatial characteristics of multiple cortical activations preceding and following saccade execution, we used the fMRI-MEG method to measure and compare neuronal activities while subjects performed both a visually-guided saccade and an apparent motion perception task. Eight healthy subjects participated in the experiments. In the normalized integrative fMRI-MEG method, time-varying dipole moments of activated regions were reconstructed from measured MEGs. Sets of activated regions were determined from statistically analyzed fMRI data and were used as spatial constraints for the integrative fMRI-MEG method. Dynamic recurrent neural activities prior to saccade onset were successfully detected in multiple cortical areas including the V1/V2, V2/V3, frontal eye field (FEF), human middle temporal area (hMT), human medial superior temporal area (hMST), intraparietal sulcus (IPS) and ventral intraparietal area (VIP). These activities lasted for the whole duration of saccade and also showed double-peak responses; before saccade onset and at saccade termination. These results demonstrated that our proposed normalized integrative fMRI-MEG method is able to reconstruct reasonable time courses of cortical activations commonly occurring in humans. In addition, these results suggest that the double-peak activities observed during saccade execution may be derived from the activities of saccade and fixation neurons. Moreover, repetitive activities in the V1/V2, V2/V3, MT, and MST indicate a possibility of feedforward process triggering discharge of FEF neurons.

Keywords: visually-guided saccade, apparent motion perception, magnetoencephalography, functional magnetic resonance imaging, normalized integrative fMRI-MEG method.

1. Introduction

Saccades are the fast eye movements that change one’s visual focus from one point to another, and are essential for accurate recognition of visual scenes because visual details are resolved best when imaged in the central fovea. The cortical network involved in saccade control is known to be composed of frontal eye field (FEF) centered structure that provides motor command and intraparietal sulcus (IPS) centered structure that modulates visual attention [1, 2]. Then, the superior colliculus (SC) that integrates visual and motor information into oculomotor signals in the brain stem receives projections from these two regions of the cerebral cortex. In addition, there are extensive feedforward and feedback projections between FEF and IPS[3, 4]. The dynamics of neuronal discharges in the brain regions involved in saccades is well known from electrophysiological experiments in monkeys [5]. However, the detailed time courses of neuronal activities in multiple cortical regions and dynamics of the network associated with the saccades in humans are not entirely established. Therefore, an important step toward elucidating these mechanisms involves obtaining more precise knowledge about the detailed time courses of activities in multiple cortical regions including FEF and IPS.

Recent neuroimaging techniques such as magnetoencephalography (MEG), positron emission tomography, near-infrared spectroscopy, and functional magnetic resonance imaging (fMRI) have become powerful tools for exploring higher brain functions. However, each technique has limited spatial or temporal resolution, which hampers our understanding of dynamic brain processes. To overcome these limitations, we have developed an integrative fMRI-MEG neuroimaging method[6]. This method can estimate the dynamic neural activities in multiple cortical areas with high spatio-temporal resolutions. Its usefulness was confirmed by a previously published simulation study [7], in which we proposed a method for the extraction of neural activities commonly observed in all subjects, using the clustering technique in the Montreal Neurological Institute and Hospital (MNI) coordinate. Hereafter, we refer to this
method as the normalized integrative fMRI-MEG method.

In this study, to validate the performance of the newly developed normalized integrative fMRI-MEG method and identify the temporal and spatial characteristics of multiple cortical activations both preceding and following saccade execution, we used fMRI and MEG to measure the brain activities of subjects performing a visually guided saccade and apparent motion perception task. By comparing the responses obtained from tasks, we investigated the dynamic brain processes involved in saccade execution after eliminating the brain activities induced by motion perception. In the source analyses, we used the proposed normalized integrative fMRI-MEG method.

2. Method

2.1 Subjects
Eight healthy males (age range: 21–35 years, mean 24.5) having normal visual, oculomotor, and vestibular functions participated in the MEG and fMRI experiments. Informed consent was obtained from all the subjects. The protocol was approved by the Ethical Committee of the Kyoto University Hospital for Physiological Science.

2.2 Visual stimuli and tasks
We obtained data during two visual perception tasks: a visually guided saccade (VGS) task and an apparent motion (AM) perception task. Figure 1 shows the configurations of the visual stimuli and the task design. In MEG experiments, stimuli were presented on a screen in front of the subject. In fMRI experiments, stimuli were projected on a screen located behind the scanner bed using a video projector. In MEG experiments, a dot was displayed at one of the four corners of a display region (6° × 6°), and the dot location was switched to one of the adjacent corners every 1100–1500 ms (a random interval). In fMRI measurements, the dot location was switched every 300–700 ms, because fMRI detects the integrated brain activities during each block and the amplitude of the hemodynamic response increases when the dot location is switched in rapid succession. A red fixation point was also displayed at the center of the display. In the visually guided saccade task, the subjects were instructed to follow the moving dot with their eyes. In the apparent motion perception task, the subjects were instructed to fixate on the red point and not to follow the moving dot. Furthermore, in the fMRI experiments, we obtained the data during rest conditions, in which the moving dot was not displayed and the subject fixated on the red point.

In the MEG experiments, we obtained event-related responses by treating the switching time as a trigger for averaging. In the fMRI experiments, a blocked design was used to maximize the signal-to-noise ratio and to identify the saccade regions. To detect brain activities, the fMRI data were acquired and compared among visually guided saccades, apparent motion perception and rest conditions for each block lasting 21 s. The activated areas detected by fMRI were used as localizers of the regions of interest.

2.3 MEG experiment
MEG data were recorded using a 306-channel whole-head system (VectorView; Elekta Neuromag, Finland) located inside a magnetically shielded room. All data were obtained at a frequency of 500.8 Hz with an online 0.1–160 Hz band-pass filter. Eye movements were recorded with vertical and horizontal electrooculograms in VGS and AM tasks. Artifacts were rejected by removing epochs with blinks. Subjects’ data were rejected from further analysis if large artifacts were present. Individual head shapes and sensor-frame coordinate system were coregistered by digitizing (Isotrak; Polhemus Navigation Sciences, USA) individual landmarks (nasion, left, and right preauricular points), the locations of which relative to sensor positions were derived based on signals provided by four head coils with a fixed spatial position relative to the landmarks. These landmarks allowed coregistration to the individual anatomical MR scans.

The raw MEG data were acquired from ~ 200 to 800 ms and were determined by trigger onset. MEG data, including trials in which subjects successfully performed VGS and AM perception tasks, were averaged across trials. In this study, we investigated the stimulus-locked response to focus on the response from the stimulus onset underlying the saccade execution. From the gradiometer responses, root sum square (RSS) values were calculated [8, 9].

Saccades were determined based on peak latencies of horizontal or vertical differential waveform of eye movements. The onset (or termination) of a saccade was
defined as the time at which the velocity of the eye first (or last) exceeded 10% of the peak value. Only epochs with saccades in the desired direction with latencies between 120 and 300 ms were included for further analysis. In the apparent motion task, we used only the trials in which subjects successfully focused on the fixation point.

2.4 Functional MRI experiment
Functional MRI measurements were performed on a 1.5-T MR imager (Vantage; Toshiba Medical Systems, Japan) using standard echo-planar imaging (EPI) with a standard radio-frequency head coil for signal transmission and reception. The acquisition parameters of the EPI sequences were as follows: TR 3 s, TE 40 ms, flip angle 90°, and voxel size 3.44 × 3.44 × 5.00 mm³.

The fMRI data were analyzed by statistical parametric mapping with SPM2 [10] software using MATLAB. Preprocessing included motion corrections and removal of linear trends from the time series. Functional 2D images were co-registered with 3D high resolution structural images. Statistical analysis was performed using a general linear model [11] by convolving the BOLD time series with a standard hemodynamic function corrected for the duration of the task block. A statistical parametric map was created and thresholded (family-wise error [FWE] corrected) for each subject. Group analysis was performed. The activated areas beyond a voxel level threshold at p < 0.001 uncorrected for multiple comparisons and within clusters of > 30 voxels were considered to be significant, and were superimposed on the high-resolution 3D anatomical images. In this study, to estimate the activated areas correlated to experimental tasks, we calculated VGS versus AM, VGS versus rest condition, and AM versus rest condition.

2.5 Normalized integrative fMRI-MEG method
Figure 2 shows the flowchart of the proposed integrative fMRI-MEG method. In the integrative fMRI-MEG method, the activated regions determined by the fMRI group analysis described above were treated as activated clusters, and we assumed that the current sources existed in the activated clusters. Our integrative method partially shares the same concept with the fMRI-constrained MEG source analysis [12] in terms of fMRI-constrained dipole procedure. However, if the activated clusters were defined on the MNI coordinates, we could not calculate the source activities from individual MEG signals. Therefore, to solve the biomagnetic inverse problem, we needed to know individual geometries of the sensor array and head. Accordingly, activated clusters in the MNI coordinates were transformed to individual head coordinates (inverse operation of normalization [13]). This transformation, referred to as renormalization, permits common clustering among all the subjects.

As spatial constraint, locations and orientations of equivalent current dipoles (ECDs) were estimated under the activated clusters by maximizing the inner product of the lead field and measured field vectors. In forward calculation, we calculated the lead field matrix using Sarvas’ equation [14], which is a simple analytic expression for magnetic fields using a spherical conductor model. The time-varying dipole moments in all voxels included in the clusters were obtained by applying a spatial-filter technique to event-related fields in VGS and AM conditions. We used the linearly constrained generalized least square method [7, 15], which was designed to achieve the best linear unbiased estimation. This method could achieve accurate reconstruction of MEG source activities even when MEG-invisible sources, which have a high temporal correlation to sources detected by fMRI, are present (for more details, see [7]). Although the plus and minus signs of reconstructed activities indicate the direction of ECD, we calculated the absolute values of reconstructed activities to simplify the source activities.

To determine the common neural dynamics related to saccades, we averaged the reconstructed source activities for all the subjects in each cluster. Hereafter, reconstructed activities imply the grand average of reconstructed activities, unless indicated otherwise. Then, to investigate the dynamic brain processes involved in saccade execution after eliminating the brain activities induced by motion perception, we subtracted the activities in AM from that in VGS. The difference in brain activation that exceeded the threshold of 5 SD for the baseline duration was considered to be a significant neural difference between VGS and AM. This threshold
was determined based on the idea of Bonferroni correction to control the familywise error rate.

3. Results

Figure 3 (a) shows the superposition of event-related fields at all sensors in both VGS and AM conditions in a representative subject. In all the subjects, prominent event-related fields (ERFs) of VGS were universally observed after approximately 200 ms at the sensors in the vicinity of the middle occipital and parietal cortices. In the AM condition, prominent ERFs were also observed at approximately 200 ms. However, the amplitudes of ERFs in VGS were larger than those in AM. Furthermore, the onset latency of saccades was 197.1±18.0 ms (mean±SD), and the termination latency was 245.4±20.1 ms.

The fMRI activation contrast for VGS versus AM yielded multiple activities in functional areas of bilateral V1/V2, V2/V3, FEF, and right ventral intraparietal area (VIP) (Fig. 3b). These areas were used as activated clusters. In addition, bilateral hMTs/MSTs, which were observed in the contrast for AM versus rest, were included in the activated clusters because hMT/MST have important roles for visual motion processing and are induced by this stimuli. Furthermore, bilateral IPSs, which were determined by the contrast of VGS versus rest, were included in the activated clusters. Thus, we identified the coordinates of the thirteen activated clusters as follows: V1/V2, V2/V3, hMT, hMST, IPS, FEF in the right and left hemispheres, and right VIP. Table 1 shows the ranges of activated clusters and the voxel numbers included in the clusters.

Figure 4 shows the activation clusters detected by fMRI group analysis. It also shows the grand mean of the time courses of reconstructed activations in the corresponding activated clusters. In the VGS condition, dynamic recurrent neural activities in multiple cortical areas were successfully detected. The neural activities in all activated clusters had multiple peaks at approximately 200 ms. The activities in the AM condition also had peaks at
approximately 200 ms, but the peak activities in VGS had larger amplitudes than those in AM over all latencies.

Figure 5 shows the differences in the grand mean reconstructed MEG source activations for the 13 activated clusters and the difference in reconstructed MEG source activation patterns on the axial MR slices. The differences in neural activities in bilateral V1/V2, V2/V3, hMT, and hMST showed peaks at approximately 168 and 220 ms, those in the left FEF at 180 and 280 ms, those in the right FEF at 180 and 244 ms, those in bilateral IPSs at 220 ms, and those in the right IPS at 244 ms. These activities lasted for the whole duration of saccade and showed double-peak responses.

4. Discussion

To perform VGS, two processes are required. One is target selection and the other is motor preparation. These saccade processes result from complex interactions between cortical (typified by FEF and IPS) and subcortical (typified by SC and reticular formation) processes, both of which play important roles [2]. In cortical processes of saccades, both IPS that modulates visual attention and FEF that provides motor command have projections to SC, so that cortical processes are crucial stages prior to subcortical processes. Although MEG is not advantageous for probing subcortical processes, it can detect cortical process associated mainly with visual attention and motor command. Besides, since a number of studies on saccades using various modalities such as fMRI, MEG, and EEG have been reported, we believe that application of the proposed method to a well known cognitive process, i.e., saccade execution, is reasonable to validate its performance.

In this study, because MEG data were recorded during the saccadic eye movements, we had to consider the influence of saccadic spike artifacts, which was localized to the region of the extraocular muscles. However, one study on the saccadic spike artifact in MEG reported that the saccadic spike artifact in MEG does not affect posterior sensors [16]. Consequently, saccadic neuromagnetic fields are not prone to misinterpretation as gamma activity reflecting higher visual processes in the parietal and occipital areas.

Since we did not differentiate the data in the four orthogonal saccadic directions, pooling the responses to saccades for four orthogonal directions might had some effects on the results. However, Tzelepi et al. [17] reported that, to a large extent, horizontal and vertical targets activated similar areas and there were no differences between activations for horizontal and vertical saccades in the stimulus-locked analysis. Thus, we expected that pooling the four orthogonal saccadic data might not negatively affect the results.

From the fMRI results, thirteen activated clusters were determined. These results are consistent with previous fMRI studies on saccades [18-21]. In the contrast of VGS versus AM, no hMT+/V5 activities were observed. This result may indicate that hMT+/V5 was activated in a manner similar to the AM perception task [22]. Although the difference of hMT+/V5 in both tasks was not observed in our fMRI analysis, there may be fMRI-invisible MEG sources that reflect the differences between two visual perception tasks. Therefore, hMT/ MST and IPS detected by different fMRI contrasts were included in the activated clusters for the subsequent integrative fMRI-MEG analyses.

For the normalized integrative fMRI-MEG method, we observed differences in neuronal activities both preceding and following saccades across IPS and FEF (which are assumed to be key areas for saccade generation) and other cortical areas along dorsal visual pathways. These results are consistent with a previous

<table>
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EEG/MEG study [23] and a previous fMRI-MEG study [24]. To perform saccade in response to a visual stimulus, sensorimotor transformation is required. One study on saccade found that the sensorimotor transformation is the product of coordinated activity across IPS and FEF, which are key components of a cortical network for saccadic generation[1]. Therefore, our results especially suggest that the difference in activity in FEF and IPS...
reflects the processes of saccade execution. In addition, a stronger response was observed in VGS condition compared with the AM condition. This could be attributed to the difference in receptive field in which each stimulus was detected.

In this study, activations in V1/V2, V2/V3, and hMT/MST were simultaneously observed preceding saccades. These results are consistent with a previous study that reported neural activities preceding VGS in the cuneus and the middle occipital area\textsuperscript{23}. Besides, neural activities in V1/V2, V2/V3, MT, MST, FEF, and IPS lasted for the duration of saccade and showed double-peak response; before saccade onset and at saccade termination. The FEF and SC are known to contain distinct populations of fixation and saccade neurons\textsuperscript{25}. Fixation neurons are tonically active during visual fixation and they cease to discharge during the execution of saccades. On the other hand, saccade neurons have a contrasting pattern of activity; they are silent during fixation and discharge a high-frequency burst of action potentials for saccades. From single neuron recording, it is known that saccade and fixation neurons in the FEF discharge at the saccade onset and termination latencies, respectively\textsuperscript{5}. Therefore, we speculate that these double-peak activities during saccade may be derived from the activities of saccade and fixation neurons. Moreover repetitive activities in V1/V2, V2/V3, MT, and MST indicate a possibility of feedforward process triggering discharge of FEF neurons. In addition, alternating neural activations in IPS and FEF; some advocate sequential response\textsuperscript{24} while others propose no difference\textsuperscript{1} for these regions. In this study, since alternating neural activations in IPS and FEF were observed, sequential responses are mediated by feedback and feedforward projections between IPS and FEF.

5. Conclusion

From the fMRI results, activated clusters such as V1/V2, V2/V3, hMT/MST, FEF, VIP, and IPS were determined on the basis of the contrasts among VGS, AM, and rest conditions. MEG results showed that VGS responses related to saccades had larger prominent ERFs than AM responses after approximately 200 ms at the sensors in the vicinity of the middle occipital and parietal cortices. Our fMRI-MEG method successfully detected dynamic cortical activities in all activated clusters. These results suggest that the double-peak activities during saccade may be derived from the activities of saccade and fixation neurons. Moreover repetitive activities in V1/V2, V2/V3, MT, and MST indicate a possibility of feedforward process triggering discharge of FEF neurons. In addition, sequential responses mediated by feedback and feedforward projections between IPS and FEF were observed. Finally, the results of this study demonstrate that the normalized integrative fMRI-MEG method is reliable and useful for reconstruction of cortical activities commonly occurring in humans, with high spatial and temporal resolutions.
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Hiroaki NATSUOKAWA

Hiroaki NATSUOKAWA was born in 1986. He received the B.S. degree in electrical and electronic engineering from Kyoto University in 2008, the M.S. in electrical engineering from Kyoto University in 2010. He is currently working toward a Ph.D. degree in electrical engineering at Kyoto University. He is also a research fellow of Japan Society for the Promotion of Science (JSPS) since April, 2010. His research interests include functional brain imaging and visual awareness.
Tetsuo Kobayashi
Tetsuo Kobayashi was born in 1956. He received the Ph.D. degree in electronic engineering in 1984, from Hokkaido University, Sapporo, Japan. He is currently a professor at Department of Electrical Engineering, Kyoto University. He was a visiting faculty member at Department of Electrical Engineering, University of Rochester, Rochester, NY from 1987 to 1988 and at Brain Behavior Laboratory, Simon Fraser University, BC, Canada, from 1996 to 1997. His research interests include brain mechanisms of binocular rivalry and human neurocortical dynamics.