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Event related fMRI studies of voluntary and inhibited eye blinking using a time marker of EOG

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Abstract

Electrooculogram (EOG) measurements, along with infrared measurements, are commonly used to record eye blinking during functional magnetic resonance imaging (fMRI). We report herein, on the use of EOG in measuring voluntary and inhibited eye blinking during echo planar imaging (EPI) in an MR scanner. The inhibited eye blinking occurred during the period, in which subjects were requested not to blink their eyes. After the removal of gradient-field induced artifacts from the EOG signal, the waveform of the EOG clearly showed both voluntary and inhibited eye blinking. Using these data, each voluntary or inhibited eye-blinking event was used as the temporal cue for an event related fMRI. Activation of the bilateral parahippocampal, precentral gyrus and left supplementary motor area was observed for voluntary eye blinking, whereas the medial/superior frontal, precentral, cingulate, precuneus, and superior temporal gyrus appears to be involved in inhibited eye blinking. Based on these experimental results, we propose that the precentral gyrus is responsible for both voluntary and inhibited eye blinking. The parietal area (precuneus and superior temporal gyrus) appears to be exclusively related to inhibited eye blinking.

Keywords: Electrooculogram (EOG); Event related fMRI; Eye blinking detection; Inhibited eye blinking; Voluntary eye blinking

The eye blink mechanism has several effects; it physically protects the cornea and promotes lubrication [4]. Most blinking occurs reflexively or spontaneously and, to some extent, regularly. However, willful and voluntary blinks can be initiated at any time by normal individuals [16]. Spontaneous, reflexive, and voluntary eye blinking in humans can be distinguished by their duration, amplitude and context. Spontaneous blinks are typically of a shorter duration than reflexive and voluntary blinks, and voluntary blinks show the greatest amplitude in the electrooculogram (EOG) waveform [10]. Reflexive blinks occur in response to an external stimulus (e.g., a sudden appearance of an object near the eyeball or a loud noise) and are related to classic conditioning [14]. The purpose of a spontaneous eye blink is to keep the eyeball in a lubricated and cleansed state. Therefore, the rate is sufficiently frequent to prevent eye from drying [8].

With the aid of recently developed imaging techniques such as functional magnetic resonance imaging (fMRI) or positron emission tomography (PET), the neural mechanisms of humans during eye blinking, especially voluntary eye blinking have been investigated [10]. In a previous study of voluntary eye blinking using fMRI, the regions of the brain activation that were activated were found to be the wide portion of the frontal gyrus and the posterior parietal cortex [4]. Kato and Miyauchi [10] reported that the precentral gyrus was responsible for the control of voluntary eye blinking. Various studies involving the human eye blink have taken advantage of infrared light reflectance to measure eyelid responses [6]. The use of reflected infrared light to measure eyelid responses has also been reported to be feasible [10,11], but the setup for this device is complicated for both technical and economical reasons. However, EOG is a suitable tool for measuring and detecting eyelid activity during functional imaging in an MR scanner. This brings more advantages compared to infrared measurement. In a related study to us [17], we have tried to pick up spontaneous eye blinking events using fMRI and EOG simultaneous measurement.

In the present study, the EOG events of eye blinking (including newly added inhibited eye blinking) were detected by means of an MR scanner. The detected blinking events were used in an analysis of event related fMRI. In the experimental design, the subjects were instructed to blink their eyes voluntarily at a given

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time point, but to inhibit eye blinking except at the given time point. EOG recordings of the eye blinking at the given time point and during the inhibition period were separately used as a temporal cue in the fMRI analysis.

Seven male and five female right-handed volunteers (mean age: 22 years, S.D.: 1.5 years) participated in the study. All of the subjects had normal or corrected-to-normal vision. They had no history of any medical, neurological or psychiatric illness in the past or at present, and they were not taking any medication.

The visual stimuli were produced using custom designed software. The stimuli were presented to the subjects by means of a video projector on a screen via a mirror. The subjects were asked to focus their view on the middle of the screen, on which a fixation point was shown. During this period they were instructed not to blink their eyes as long as the fixation point was on the screen. After the presentation of a fixation point for 20, 25, or 30 s, the fixation point was disappeared for 500 ms, in which the subjects could blink their eyes voluntarily. This presentation pattern was repeated 15 times for each subject. The interstimulus interval of 20, 25, and 30 s was repeated randomly. The total imaging time for an experimental run was 6 min and 27.5 s for each subject.

An fMRI-compatible electroencephalogram recording system, BrainAmp-MR (Brain Products GmbH, Munich, Germany) with a specially designed electrode cap (BrainCap-MR), was used for the detection and recording of eye blinking during MR imaging. The electrode cap included 32 electroencephalogram channels and three additional channels of one electrocardiogram (EKG) and two electrooculograms (EOG). All electrodes were ring-typed sintered nonmagnetic Ag/AgCl electrodes. The reference channel in the cap was located at the center of the subject's forehead. The impedance of each electrode site was maintained at less than 5 KΩ by injecting electrode paste (ABRALYT 2000, FMS, Herrsching-Breitbrunn, Germany). The EOG electrode was positioned 1 cm below the right eye of each subject. The EOG and EKG signals from the electrode were transferred to the amplifier via a nonferrous ribbon cable. The amplifier was designed so as to be placed inside the MR scanner and was connected to a computer in the operating room via an optical fiber cable. The precision and dynamic ranges of the amplifier were 100 nV and ±3.3 mV, respectively. EOG waveforms were recorded at a sampling rate of 500 samples/s. Bandpass filtering from 0.5 to 80 Hz was applied in conjunction with 60 Hz notch filtering.

Before starting a scanning session, an EOG waveform of each subject was recorded as reference data. The correction of gradient-field induced artifacts was performed using the commercially available software, Vision Analyzer (Brain Products GmbH, Munich, Germany) [2]. After removing gradient-field induced artifacts from the EOG recording, the eye-blinking signal could be clearly identified. This waveform was over 150 μV, with a frequency of about 2–4 Hz. However, it was difficult to differentiate the EOG waveforms corresponding to voluntary and inhibited eye blinking, since they were similar. Therefore, it is quite important to record the time marker of each echo planar imaging (EPI) pulse sequence during the recording of the EOG, as shown in Fig. 1.

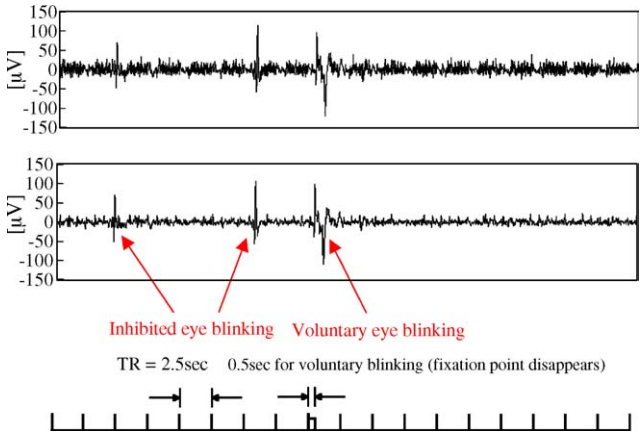

Fig. 1. EOG waveforms for the voluntary and inhibited eye blinking of one subject. Top: the voluntary and inhibited eye blinking waveform during MR imaging. Middle: the waveform after gradient-field induced artifacts correction. Bottom: time marker for the EPI pulse sequence and voluntary eye blinking.

Images were acquired using a KAIST 3 T MRI scanner (ISOL Technology, Korea) with a quadrature head coil. Following a T1-weighted scout image, high-resolution anatomic images were acquired using an MPRAGE (Magnetization-Prepared Rapid Gradient Echo) pulse sequence with TR = 8.1 ms, TE = 3.7 ms, flip angle = 8°, and an image size of 256 × 256. T2*-weighted functional data were acquired using an EPI pulse sequence of TE = 37 ms, flip angle = 70°, TR = 2500 ms, and an image size of 64 × 64. We obtained 30 slices of EPI images with a slice thickness of 5 mm, with no gaps between the slices, for the entire brain. A total of 155 volumes were acquired per experimental run. For each subject, the first five volumes in each scan series were discarded, since they were collected before the magnetization had reached the equilibrium state.

Image data were analyzed using SPM99 (Wellcome Department of Cognitive Neurology, London). The images for each subject were corrected for motion and were realigned using the first scan as a reference. T1 anatomical images were coregistered with the mean of the functional scans (EPI images) and then normalized to the SPM T1 template in the atlas space of Talairach and Tournoux [15]. Finally, the images were smoothed by applying a Gaussian filter of 7 mm full-width at half-maximum (FWHM). The voluntary and inhibited eye blinking events were subsequently modeled (event related design) using a canonical hemodynamic response and its temporal derivative [9]. Planned comparisons were computed as contrasts for each individual participant. The resulting contrast images were then used in random effect analyses at the group level. Activations were reported if the P value satisfied with $P < 0.001$ (uncorrected at the single voxel level). Activations were based on the extent of 10 voxels.

The subjects were asked to blink their eyes voluntarily when the fixation point disappeared. This was monitored by EOG recordings. The EOG data provided confirmation as to whether the subjects blinked their eyes only at the time when a fixation point disappeared. The whole subjects blinked their eyes voluntarily as indicated. The blinks that did not correspond to the time when the fixation point disappeared were considered to be an inhibited eye-blinking event. The recorded voluntary

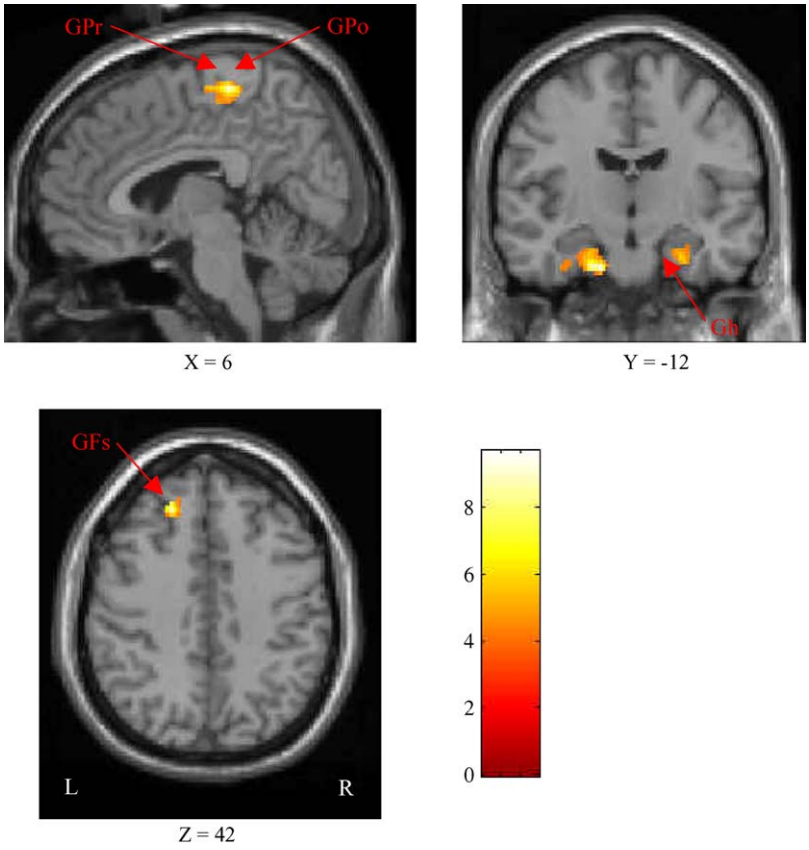

Fig. 2. Map of the activation area for voluntary eye blinking: GPr, precentral gyrus; GPo, postcentral gyrus; Gh, parahipocampal gyrus; GFs, superior frontal gyrus.

and inhibited eye blinking events were the temporal cue for the data. A cerebral activation pattern of voluntary eye blinking at the group level was observed in the bilateral parahippocampal gyrus (BA 28), precentral gyrus (BA 6), and left hemispheric superior frontal gyrus (BA 8), as shown in Table 1 and Fig. 2.

The cerebral activation pattern of inhibited eye blinking at the group level was observed in the left hemispheric superior frontal gyrus (BA 6), right hemispheric medial frontal gyrus (BA 6), left hemispheric precentral gyrus (BA 4), right hemispheric fusiform gyrus (BA 37), right hemispheric precuneus (BA 19), right hemispheric superior temporal gyrus (BA 22) and right hemispheric cingulate gyrus (BA 32), as shown in Table 2 and Fig. 3.

The EOG, as a recording system, permits the detection of eye blinking during MR imaging. On the basis of this recording, each eye-blinking event could be considered for a functional analysis. The recording of each eye-blinking event is not only meaningful for the data analysis of event related designs, but it is also used to monitor the subjects as to whether they precisely followed the experimental protocol. Furthermore, according to this monitoring mechanism, it is possible to observe whether it is difficult for some subjects to follow the experimental protocol during the scanning session, since looking at a fixation point for more than 6 min is a difficult task. For this reason, several eye blinking events occurred during the inhibition period.

Bilateral parahippocampal activation was found to be associated with voluntary eye blinking. This finding is of interest, since it was assumed that this activation was not directly involved in voluntary eye blinking. According to previous studies, parahippocampal activation is involved with spatial memory [13]. Patients with lesions involving the parahippocampal cortex suf-

Table 1
Activation area for voluntary eye blinking ($P < 0.001$, uncorrected at the single voxel level, $P < 0.05$ corrected at the cluster level)

Cerebral area	BA, hemispheric side	x, y, z (mm)	Z-value
Parahippocampal gyrus	28L	−22, −12, −30	4.89
Parahippocampal gyrus	28R	24, −16, −20	4.31
Superior frontal gyrus	8L	−22, 36, 42	4.67
Precentral gyrus	6/4L	−6, −28, 64	4.77
Precentral gyrus	6/4R	6, −22, 66	4.45

BA: Brodmann area.

Table 2
Activation area for inhibited eye blinking ($P < 0.001$, uncorrected at the single voxel level, $P < 0.05$ corrected at the cluster level)

Cerebral area	BA, hemispheric side	x, y, z (mm)	Z-value
Medial frontal gyrus	6R	32, −4, 50	4.88
Superior frontal gyrus	6L	−10, −8, 48	4.00
Precentral gyrus	4L	−28, −18, 50	3.92
Fusiform gyrus	37R	52, −60, −10	4.55
Precuneus	19R	26, −78, 38	4.24
Superior temporal gyrus	22R	48, −34, 4	4.58
Cingulate gyrus	32R	16, 0, 46	4.43

BA: Brodmann area.

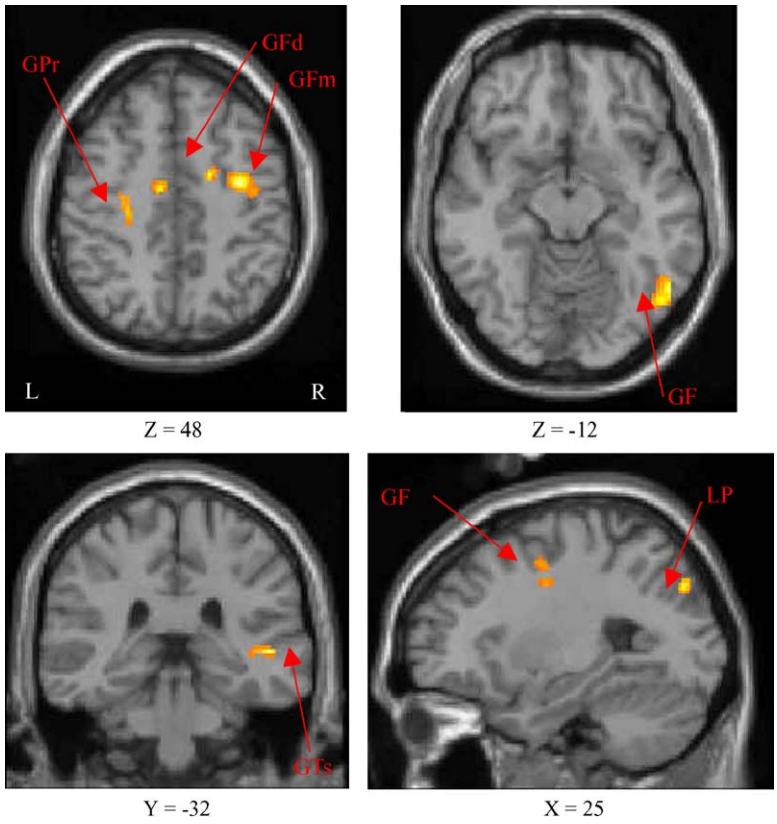

Fig. 3. Map of the activation area for inhibited eye blinking: GPr, precentral gyrus; GFd, medial frontal gyrus; GFm, middle frontal gyrus; GF, fusiform gyrus; GTs, superior temporal gyrus; LPs, superior parietal lobule.

fer from an anterograde topographical disorientation syndrome, i.e., impairment in the ability to acquire topographical information for navigation in previously unfamiliar environments [3]. The possible cognitive deficits underlying this syndrome are probably manifold, since real-world navigation is a complex cognitive task that requires the integration of spatial relationships and landmarks of the environment with the actual body coordinates of the subject [1]. In this context, the parahippocampal cortex has been related to the representation of allocentric space. Parahippocampal lesions may affect some basic functions that are relevant to allocentric forms of spatial memory [13]. This suggests that the parahippocampal activation found here might be related with this allocentric form of spatial memory. In the experimental protocol, the subjects were requested to blink their eyes voluntarily when a fixation point disappeared, whereas they should fix their view on the fixation point for more than 20 s. Even though the disappearance of the fixation point for 500 ms was the marker for voluntary eye blinking, it appears that the subjects needed spatial memory for maintaining the location of the fixation point during the blinking period.

Cerebral activations of the bilateral precentral gyrus for voluntary eye blinking and the left precentral gyrus for inhibited eye blinking were observed. The relationship between the activation of this area and eye blinking is not clear. Bruce et al. [5] reported in their study with monkeys that blinks were often evoked by stimulation in the posterior bank of the arcuate sulcus, in which arm and hand movements or mouth movements are also often evoked by stimulation. However, eye movements were not evoked by stimulation of the bilateral precentral gyrus. In contrast, stimulation in the anterior area to the frontal eye field evoked saccades at a high threshold or failed to elicit any movements at all. These results indicate that the eye blinking area is located posteriorly, not anteriorly, to the frontal eye field in monkeys, and that the eye blinking area is included in the premotor area, which is related to skeletomotor control rather than in the oculomotor area. Kato and Miyauchi [10] suggested in their study of intentional eye blinking, that the activation area of the precentral gyrus corresponded to the premotor area in humans. On the basis of our results, we suggest that precentral activation might be related to both voluntary eye blinking and inhibited eye blinking. Activation of the cingulate gyrus, which is located close to the cingulated/frontal eye field, also appears to be involved.

Additional activation of the superior frontal gyrus for voluntary eye blinking was observed as well. Previous studies have indicated that the superior frontal area might correspond to the supplementary motor area and the involvement of the supplementary motor area in self-paced movement has been proposed [7]. It seems that our results of voluntary eye blinking might be due to the general involvement of supplementary motor area in self-paced movement. On the basis of this, further activation patterns of the superior/medial frontal gyrus for the inhibited eye blinking is not easy to interpret. The key issue in the interpretation is whether inhibited eye blinking mechanism is self-paced

or not. We assume that inhibited eye blinking is also self-paced, because the subjects blinked their eyes due to dryness and this involves a self-control (paced) mechanism.

The additional activation of the right hemispheric fusiform, precuneus, and superior temporal gyrus was observed for inhibited eye blinking. This activation area is located close to the parietal eye field area, which is involved in the control of saccades as well as in attentional processes [12]. Inhibited eye blinking occurred due to the dry eyes of the subjects and this occurred, even during the concentration period, in which the subjects intentionally inhibited their eye blinking. We propose that this behavioral mechanism is related to the aforementioned role of the parietal eye field due to eye movement.

In conclusion, EOG events of eye blinking were detected in an MR scanner. The detected eye blinking events were used in an analysis of event related fMRI. The recorded EOG of eye blinking at a given time point and during the inhibition period were separately used as a temporal cue in the fMRI analysis. The findings suggest that, in the precentral gyrus, both voluntary and inhibited eye blinking appear to be related to the general involvement of the supplementary motor area in self-paced movement. The findings also suggest that parahippocampal activation in the case of voluntary eye blinking might be related to this allocentric form of spatial memory. Lastly, it appears that the parietal area (precuneus and superior temporal gyrus) are be related only to inhibited eye blinking.

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