Tohoku J. Exp. Med., 2016, **240**, 141-146

Cerebral Functional Response during Eyelid Opening/Closing with Bell's Phenomenon and Volitional Vertical Eye Movements in Humans

Yukihisa Suzuki,[1, 2, 3] Motohiro Kiyosawa,[1,4] Kiichi Ishiwata,[2, 5] Keiichi Oda[2, 6] and Kenji Ishii[2]

[1]Department of Ophthalmology and Visual Science, Tokyo Medical and Dental University Graduate School, Tokyo, Japan
[2]Research Team for Neuroimaging, Tokyo Metropolitan Institute of Gerontology, Tokyo, Japan
[3]Department of Ophthalmology, Japan Community Health Care Organization, Mishima General Hospital, Mishima, Shizuoka, Japan
[4]Kiyosawa Eye Clinic, Tokyo, Japan
[5] Institute of Cyclotron and Drug Discovery Research, Southern Tohoku Research Institute for Neuroscience, Koriyama, Fikushima, Japan
[6]Department of Radiological Technology, Faculty of Health Sciences, Hokkaido University of Science, Sapporo, Hokkaido, Japan

Bell's phenomenon is a physiological phenomenon wherein the eye ball involuntarily rolls upward during eyelid closing. Although this phenomenon occurs in healthy individuals, the neural mechanism related to Bell's phenomenon has not yet been identified. We aimed to investigate the brain regions relevant to Bell's phenomenon and volitional eye movement using [^{15}O] H_2O and positron emission tomography (PET). We measured regional cerebral blood flow (rCBF) in 8 normal subjects under 3 conditions: at rest with eyes closed, during opening and closing of the eyelids in response to sound stimuli (lid opening/closing), and during vertical movement of the eyes with lids closed in response to sound stimuli (volitional eye movement). The supplementary motor area (SMA) proper, right superior temporal gyrus, right insular cortex and left angular gyrus were activated during lid opening/closing. The right frontal eye field (FEF), pre-SMA, left primary motor area, right angular gyrus, and SMA proper were activated during volitional eye movement. The SMA proper was active during both tasks, while the FEF and pre-SMA were active during volitional eye movement, but not during eyelid opening/closing. A comparison of activation during volitional eye movements and lid opening/closing tasks revealed a relative increase in rCBF in the FEF. There were no areas that are activated in relation to Bell's phenomenon. In conclusion, activation in the FEF mainly occurs during volitional eye movement. Since Bell's phenomenon is a reflexive eye movement, the FEF is scarcely concerned in Bell's phenomenon.

Keywords: Bell's phenomenon; frontal eye field; positron emission tomography; regional cerebral blood flow; volitional eye movement
Tohoku J. Exp. Med., 2016 October, **240** (2), 141-146.

Introduction

The eyeballs involuntarily roll upward, when humans close their eyelids (Bour et al. 2000). This is called Bell's phenomenon, and it presumably involves brainstem pathways between the seventh nerve nucleus in the pons and the third nerve nuclear complex in the rostral midbrain (Graser 1999). Bell's phenomenon is an important physical sign in clinical medicine. An impairmed Bell's phenomenon is can indicate voluntary eyelid movement disorders, including voluntary eyelid closure palsy and cerebral ptosis, which are related to lesions of frontal cortical areas and/or the corticospinal system (Esteban et al. 2004). While Bell's phenomenon certainly occurs in healthy individuals, Bell's phenomenon is hardly known outside of ophthalmology. Previous studies used magnetic search coils or electroencephalograms to observe eye movement due to Bell's phenomenon during eyelid closure and blinking (Takagi et al. 1992; Iwasaki et al. 2005). However, no report discussing the brain regions involved in Bell's phenomenon exists. Furthermore, the exact neural mechanism responsible for this integrated movement is unknown. Our aim was to

Received July 25, 2016; revised and accepted September 21, 2016. Published online October 8, 2016; doi: 10.1620/tjem.240.141.
Correspondence: Yukihisa Suzuki, M.D., Ph.D., Department of Ophthalmology and Visual Science, Tokyo Medical and Dental University Graduated School, 1-5-45 Yushima, Bunkyo-ku, Tokyo 113-8519, Japan.
e-mail: suzuki@f8.dion.ne.jp

investigate the areas of the brain related to Bell's phenomenon.

We hypothesized that the brain areas related to eye movement during Bell's phenomenon would be different from those related to volitional eye movement, as Bell's phenomenon is a reflexive eye movement. Previous studies have shown that often times, blinking does not accompany Bell's phenomenon (Takagi et al. 1992; Iwasaki et al. 2005); therefore, we adopted a lid opening/closing movement instead of blinking in the present study. To test our hypothesis, we conducted a positron emission tomography (PET) activation study comparing lid opening/closing and volitional vertical eye movement.

Materials and Methods

Subjects

Eight healthy volunteers (4 men and 4 women; age 20 to 24 years) participated in the study. None of the subjects had a history of neurologic or psychiatric disorders. All measurements in this study were performed in the Tokyo Metropolitan Institute of Gerontology. All subjects volunteered and were informed of the benefits and risks of the study, which included some radiation exposure. Informed consent was obtained from all subjects before participation in this study. This study protocol was approved by the Institutional Ethics Committee of the Tokyo Metropolitan Institute of Gerontology. All procedures conformed to the tenets of the Declaration of Helsinki.

Acquisition of positron emission tomography data

We acquired PET scans using a SET 2400W scanner (Shimadzu, Kyoto, Japan). After a transmission scan with a $^{68}Ga/^{68}Ge$ rotating source for attenuation correction, each task began 30 s before the intravenous administration (i.v.) of a bolus of 150 MBq [^{15}O] H_2O, and a 2-min emission-scan was started when activity was observed in the brain (Suzuki et al. 2010). No arterial blood was sampled and the images obtained were treated as representative of tissue activity. We measured regional tissue activity with short scanning times of 40-60 s, which has been shown to correlate almost linearly with regional cerebral blood flow (rCBF) (Fox and Mintun 1989).

The scanning room was dimmed during the PET scan, and all subjects were blindfolded to prevent any influence from the external lighting (Suzuki et al. 2010). The blindfold did not press against the eyes/eyelids or disturb lid opening/closing and eye movement. Lid opening/closing and eye movements were recorded using electrooculography (EOG) (Fig. 1). We recorded subjects during the scan with a video camera, and confirmed that there were no extra movements of the face or other body parts that could have influenced the experiment (Suzuki et al. 2010).

Magnetic resonance imaging scanning

All participants underwent a magnetic resonance imaging (MRI) scan with a 1.5-Tesla scanner (Signa Horizon; General Electric, Milwaukee, WI, USA). We obtained trans-axial images with T1-weighted contrast (three-dimensional spoiled gradient recalled acquisition in steady state [3DSPGR], TR = 9.2 ms; TE = 2.0 ms; matrix size = 256 × 256 × 124; voxel size = 0.94 × 0.94 × 1.3 mm) and T2-weighted contrast MRI (First Spin Echo, TR = 3,000 ms; TE = 100 ms; matrix size = 256 × 256 × 20; voxel size = 0.7 × 0.7 × 6.5 mm) (Suzuki et al. 2010). We confirmed that subjects did not have any organic brain disorders using T1-weighted contrast and T2-weighted contrast MR images. T1-weighted contrast MR images were used for further image processing (Suzuki et al. 2010).

Task design

The subjects performed 3 conditions in the scanner, and each condition was scanned twice: [1] at rest with eyes closed (rest), [2] lid opening/closing in response to sound stimuli (lid opening/closing), and [3] vertical movement of the eyes with lids closed in response to sound stimuli (volitional eye movement). Vertical eye movements occur during condition [2] and [3]. Each task began 30 s before the start of PET scanning (Suzuki et al. 2010). The task order was counterbalanced to prevent an order effect.

Rest. The subjects lay in a supine position on the bed of the PET scanner. We instructed them to remain stationary and not move their eyelids or any other part of their body with their eyes closed (Suzuki et al. 2010). They were asked not to think of any movement-related images or any other sensory perceptions during the scans.

Lid opening/closing. Subjects were instructed to open their eyes when they heard a personal computer (PC)-generated sound (0.2 Hz) (Suzuki et al. 2010). Following the sound, they closed their eyes immediately, and kept them closed until they heard the sound again. The sound for the task was randomized to appropriate timing using PC.

Volitional eye movement. The subjects rolled their eyes upward upon hearing the tone and then immediately downward; the subjects then, rested until they heard the next tone without opening their eyes. The intervals between sounds were randomized to prevent subjects from anticipating the timing of the sound; the frequency was the same as that used in the lid opening/closing task (Suzuki et al. 2010).

Data processing and statistical analyses

The MRIs were spatially normalized to a standard anatomical space (MNI space) using a template produced by the Montreal Neurological Institute (MNI) (Talairach and Tournoux 1988) and the normalized MRIs were used for further processing of the PET images. Unless otherwise indicated, we processed and analyzed all PET images using Statistical Parametric Mapping (SPM 8; Welcome Department of Cognitive Neurology, London, UK), implemented in MATLAB version 7.1 (Mathworks Inc., Sherborn, MA, USA) (Friston et al. 1991). The series of scanned images from each subject were realigned using the least squares approach in order to remove effects due to head motion (Suzuki et al. 2010). All PET images were registered three-dimensionally to the individual three-dimensional MRIs using SPM. All PET images were normalized using the transformation matrix of the co-registered MRI and smoothed with a 10 mm full-width half-maximum (FWHM) Gaussian kernel in the x-axis, y-axis, and z- axis (Suzuki et al. 2010).

We estimated the condition effects according to the general linear model at each voxel after the relevant design matrix was specified. The design matrix contained global activity as a confounding covariate, and this analysis can be considered as an analysis of covariance (ANCOVA) (Friston et al. 1990). In order to investigate regions of the brain related to each condition, we examined contrast images of (A) lid opening/closing minus rest, and (B) volitional eye movement minus rest. Then, to examine the differences between lid opening/closing and volitional eye movement, we used contrast images of (C) lid opening/closing minus volitional eye movement. Similarly, contrast images of (D) volitional eye movement minus lid opening/clos-

ing were examined. For all reported activated regions, the significance level exceeded $P < 0.05$, which was corrected with a 30-voxel of spatial extent threshold false discovery rate (FDR) (Suzuki et al. 2010). The resulting set of voxel values for each contrast image was used to construct a statistical parametric map of the *t* statistic, SPM (t). The location of each focus was determined as the center of region for that focus. The x-coordinates, y-coordinates, and z-coordinates of the center of region were calculated in Talairach coordinates (Talairach et al. 1988).

Results

Electrooculography

An electrooculogram is a record of the difference in electrical potential between the anterior and posterior regions of the eye, which can be used to indicate eye movement and is obtained by electrodes placed on the skin near the eye. Typical EOG data are shown in Fig. 1. However, the present data is not an average of the data from multiple subjects. While resting, the subjects reflexively blinked when their eyes were open prior to the PET scan. Reflex blinking accompanied downward eye movement, but not upward eye movement. Conversely, upward eye movements (Bell's phenomenon) were recorded during lid opening/closing tasks. Upward and downward vertical eye movements were recorded during volitional eye movement.

Comparison between the 2 motor conditions and rest

An increase in rCBF was observed in the supplementary motor area (SMA) proper, right superior temporal gyrus, right insular cortex, and left angular gyrus during lid opening/closing compared to that during rest (Fig. 2, Table 1). The rCBF increased in the right frontal eye field (FEF), pre-SMA, left primary motor area (M1), right angular gyrus, and SMA proper during volitional eye movement in comparison with the rest condition (Fig. 2, Table 1).

Comparison between 2 motor conditions

No difference in rCBF was observed upon analysis of lid opening/closing minus volitional eye movement images (Fig. 2, Table 1). However, analysis of volitional eye movement minus lid opening/closing images showed higher rCBF in the left FEF (Fig. 2, Table 1).

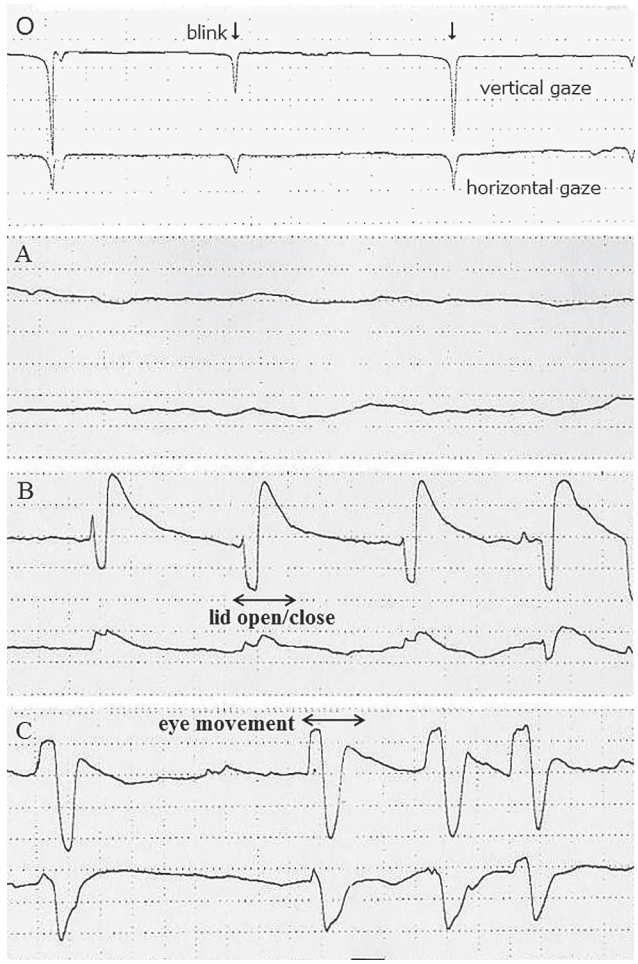

Fig. 1. Electrooculogram during each condition.
Electrooculographic recordings were conducted before the experiments (O) and during each condition (A-C). The upper line represents the vertical gaze and the lower line represents the horizontal gaze.
(O) At rest with eyes open. Subject opened their eyelids. Reflexive blinking was recorded every few seconds. Downward eye movements were recorded during blinking.
(A) Rest: Eyes closed. There were no major eye movements or blinking.
(B) Lid opening/closing: Subjects opened and closed their eyes in response to certain tones. Upward eye movements were recorded after downward eye movements during lid opening/closing.
(C) Volitional eye movement: With their eyelids shut, the subjects rolled their eyes upward upon hearing the tones, and then downward.

Discussion

We compared cerebral activation between lid opening/closing and volitional eye movement, in order to investigate the mechanism driving Bell's phenomenon. An increase in the relative rCBF in the SMA proper was observed during both tasks: however, the FEF was activated only during volitional eye movement. Comparison of volitional eye movement and lid opening/closing revealed a relative rCBF increase in the FEF.

Bell's phenomenon and blinking

Blinking may accompany Bell's phenomenon. However, previous studies have observed that upward eye movements did not occur during blinking. Takagi et al. (1992) used the magnetic search coil method to study eye movements associated with spontaneous blinks, voluntary blinks and prolonged eyelid closure. They observed that spontaneous blinks and voluntary blinks were accompanied by small downward and nasalward eye movements, while prolonged eyelid closure was accompanied by upward movement. Moreover, Iwasaki et al. (2005) observed that spontaneous blinks did not produce Bell's phenomenon,

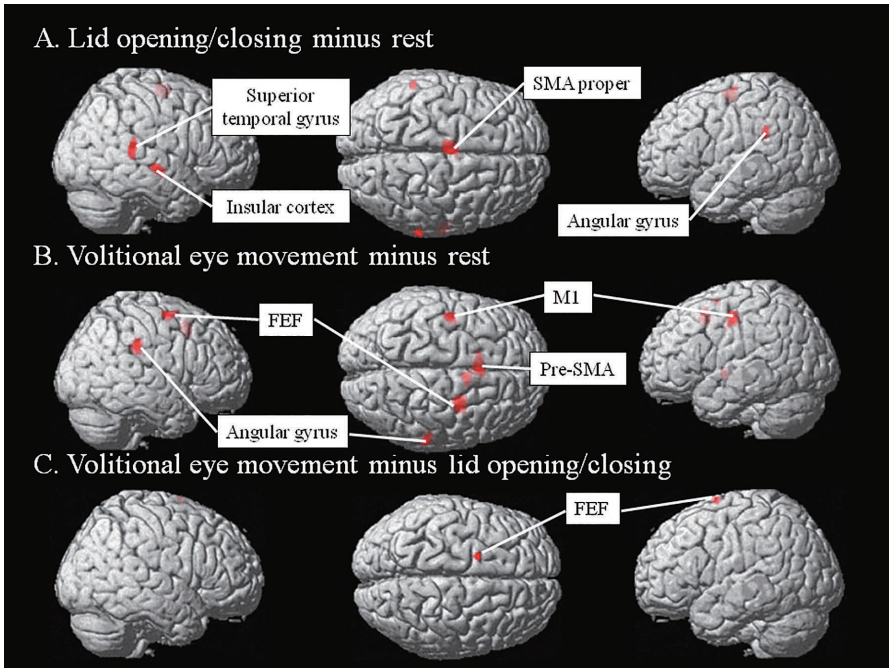

Fig. 2. Subtraction images between each condition.
Lateral and superior views of a statistical parametric map (SPM) rendered into standard stereotactic space ($P < 0.05$, false discovery rate [FDR]-corrected).
(A) Lid opening/closing minus rest: The areas where cerebral blood flow increased during "Lid opening/closing" condition compared with "rest" condition are shown. The supplementary motor area (SMA) proper, superior temporal gyrus, insular cortex, and angular gyrus were activated.
(B) Volitional eye movement minus rest: The areas where cerebral blood flow increased during "volitional eye movement" compared with "rest" were shown. The frontal eye field (FEF), pre-SMA, primary motor area (M1), angular gyrus, and SMA proper were activated.
(C) Volitional eye movement minus lid opening/closing: The areas where cerebral blood flow increased during "volitional eye movement" condition compared with "Lid opening/closing" condition are shown. The FEF was activated.
Extent threshold, k = 30 voxels.

whereas slow or forced blinks were associated with delayed upward eye rotations. From these observations, we suspected that prompt blinking does not accompany Bell's phenomenon.

Cerebral blood flow differences between lid opening/closing and volitional eye movement

We observed that the SMA proper was activated during both tasks; in contrast, the pre-SMA was activated during volitional eye movement, but not during lid opening/closing. These 2 areas belong to the medial portion of the premotor cortex. The SMA proper is located posterior to the vertical line traversing the anterior commissure (VCA line); the pre-SMA, anterior to the VCA line (Picard and Strick 1996). These areas are mainly engaged in the preparation and processing for voluntary movements (Schubotz and von Cramon 2001). Recent brain PET imaging studies in humans showed that the SMA proper might be activated during the generation of simple repetitive movements, whereas the pre-SMA is activated during more complex motor sequence tasks (Picard and Strick 1996). Our results indicated that the SMA proper was activated more during eye opening/closing and during volitional eye movement than during rest. We suspect that the human brain regards eye movements as more complex than eye opening/closing because eye movement without opening the eyelids is unnatural and difficult.

We observed that the FEF was only activated during volitional the eye movement, but not during lid opening/closing. The FEF, supplementary eye field (SEF), and parietal eye field (PEF) are all areas of the cerebral cortex related to eye movements (Pierrot-Deseilligny et al. 1995). The SEF is located on the medial surface of the superior frontal gyrus in the upper part of the paracentral sulcus, and the SEF is involved in motor programs comprising a saccade combined with a body movement or in a sequence of several successive saccades. The PEF is involved in the control of saccades and in attentional processes. The FEF is located around the lateral part of the precentral sulcus and gyrus, immediately anterior to the motor cortex. Generally, the FEF is activated immediately before saccadic eye movement (Bruce and Goldberg 1985). Cassanello and Ferrea (2007) observed that signals related to eye position activated the FEF in monkeys. Moreover, the FEF was reportedly activated by fixation, visual stimuli, and during the initiation of eye movements, despite an eye movement not being performed (Hanes et al. 1998). In the present study, we observed that the right FEF was only activated

Table 1. Areas and coordinates of maximal regional cerebral blood flow (rCBF) increases.

Area activated	x	y	z	Z score	Cluster size
Lid opening/closing vs. rest					
SMA proper	–8	–12	68	4.08	147
Superior temporal gyrus (R)	68	–38	10	3.87	75
Insular cortex (R)	64	–16	-4	3.79	77
Angular gyrus (L)	–58	–42	28	3.58	43
Volitional eye movement vs. rest					
FEF (R)	30	–4	62	4.34	84
Pre-SMA	–2	12	48	4.14	157
M1 (L)	–44	–12	48	3.75	96
Angular gyrus (R)	60	–30	34	3.69	80
SMA proper	8	4	64	3.56	37
Lid opening/closing vs. volitional eye movement					
None					.
Volitional eye movement vs. lid opening/closing					
FEF (L)	–18	2	74	3.66	40

Areas with Z > 3.25 ($P < 0.05$, FDR-corrected) are listed.
Global cerebral blood flow = 50.
SMA, supplementary motor area; FEF, frontal eye field; M1, primary motor area; R, right side; L, left side.

during volitional eye movement, but not during lid opening/closing tasks. Several studies reported that the FEF is not activated during eye blinking (Schreurs et al. 2001; van Eimeren et al. 2001). Conversely, Bodis-Wollner et al. (1999) reported that the FEF was activated during voluntary saccades and voluntary blinking; however, fewer voxels were activated during blinking than during saccades. Though they did not mention whether Bell's phenomenon was observed during blinking, we suspect that it was not generated in their study. The results of these studies may be because of concomitant small volitional eye movements or the initiation of eye movement during blinks because prompt blinks do not accompany Bell's phenomenon (Takagi et al. 1992; Iwasaki et al. 2005). These observations indicate that Bell's phenomenon is not accompanied by the FEF activation in many cases.

Conclusions

There are no noticeable brain areas that are activated in relation to Bell's phenomenon. As Bell's phenomenon is a reflexive eye movement, it is not surprising to find that the FEF is not involved in Bell's phenomenon. By contrast, the FEF is mainly concerned with volitional eye movement.

Conflict of Interest

The authors declare no conflict of interest.

Acknowledgments

This study was supported by a Grants-in-Aid for Scientific Research (B) 21791725 (Y.S.) from the Japan Society for the Promotion of Science.

References

Bodis-Wollner, I., Bucher, S.F. & Seelos, K.C. (1999) Cortical activation patterns during voluntary blinks and voluntary saccades. *Neurology*, **53**, 1800-1805.

Bour, L.J., Aramideh, M. & de Visser, B.W. (2000) Neurophysiological aspects of eye and eyelid movements during blinking in humans. *J. Neurophysiol*., **83**, 166-176.

Bruce, C.J. & Goldberg, M.E. (1985) Primate frontal eye fields. I. Single neurons discharging before saccades. *J. Neurophysiol*., **53**, 603-635.

Cassanello, R.C. & Ferrea, V.P. (2007) Computing vector differences using a gain field-like mechanism in monkey frontal eye field. *J. Physiol.*, **582**, 647-664.

Esteban, A., Traba, A. & Prieto, J. (2004) Eyelid movements in health and disease. The supranuclear impairment of the palpebral motility. *Neurophysiol. Clin.*, **34**, 3-15.

Fox, P.T. & Mintun, M.A. (1989) Noninvasive functional brain mapping by change-distribution analysis of averaged PET images of $H_2{}^{15}O$ tissue activity. *J. Nucl. Med*., **30**, 141-149.

Friston, K.J., Frith, C.D., Liddle, P.F., Dolan, R.J., Lammertsma, A.A. & Frackowiak, R.S.J. (1990) The relationship between

global and local changes in PET scans. *J. Cereb. Blood Flow Metab*., **10**, 458-466.

Friston, K.J., Frith, C.D., Liddle, P.F. & Frackowiak, R.S. (1991) Comparing functional (PET) images: the assessment of significant change. *J. Cereb. Blood Flow Metab*., **11**, 690-699.

Graser, J.S. (1999) *Neuro-ophthalmology*, 3rd ed. J. B. Lippincott Company, Philadelphia.

Hanes, D.P., Patterson, W.F. 2nd. & Schall, J.D. (1998) Role of frontal eye fields in countermanding saccades: visual, movement, and fixation activity. *J. Neurophysiol.*, **79**, 817-834.

Iwasaki, M., Kellinghaus, C., Alexopoulos, A.V., Burgess, R.C., Kumar, A.N., Han, Y.H., et al. (2005) Effects of eyelid closure, blinks, and eye movements on the electroencephalogram. *Clin. Neurophysiol.*, **116**, 878-885.

Picard, N. & Strick, P.L. (1996) Motor areas of the medial wall: a review of their location and functional activation. *Cereb. Cortex*, **6**, 342-353.

Pierrot-Deseilligny, C., Rivaud, S., Gaymard, B., Muri, R. & Vermersch, A.I. (1995) Cortical control of saccades. *Ann. Neurol.*, **37**, 557-567.

Schreurs, B.G., Bahro, M., Molchan, S.E., Sunderland, T., Anthony, R. & McIntosh, A.R. (2001) Interactions of prefrontal cortex during eyeblink conditioning as a function of age. *Neurobiol. Aging*, **22**, 237-246.

Schubotz, R.I. & von Cramon, D.Y. (2001) Functional organization of the lateral premotor cortex: fMRI reveals different regions activated by anticipation of object properties, location and speed. *Brain Res. Cogn. Brain Res.*, **11**, 97-112.

Suzuki, Y., Kiyosawa, M., Mochiuki, M., Ishiwata, K. & Ishii, K. (2010) The pre-supplementary and primary motor areas generate rhythm for voluntary eye opening and closing movements. *Tohoku J. Exp. Med.*, **222**, 97-104.

Takagi, M., Abe, H., Hasegawa, S. & Usui, T. (1992) Reconsideration of Bell's phenomenon using a magnetic search coil method. *Doc. Ophthalmol.*, **80**, 343-852.

Talairach, J. & Tournoux, P. (1988) Co-planar stereotaxic atlas of the human brain. *3-dimentional proportional system: An approach to cerebral imaging*, Thieme Medical Publishers, Stuttgart.

van Eimeren, T., Boecker, H., Konkiewitz, E.C., Schwaiger, M., Conrad, B. & Ceballos-Baumann, A.O. (2001) Right lateralized motor cortex activation during volitional blinking. *Ann. Neurol*., **49**, 813-816.