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The Representation of Blinking Movement in Cingulate Motor Areas: A Functional Magnetic Resonance Imaging Study

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Recent anatomical evidence from nonhuman primates indicates that cingulate motor areas (CMAs) play a substantial role in the cortical control of upper facial movement. Using event-related functional magnetic resonance imaging in 10 healthy subjects, we examined brain activity associated with volitional eye closure involving primarily the bilateral orbicularis oculi. The findings were compared with those from bimanual tapping, which should identify medial frontal areas nonsomatotopically or somatotopically related to bilateral movements. In a group-level analysis, the blinking task was associated with rostral cingulate activity more strongly than the bimanual tapping task. By contrast, the bimanual task activated the caudal cingulate zone plus supplementary motor areas. An individual-level analysis indicated that 2 foci of blinking-specific activity were situated in the cingulate or paracingulate sulcus: one close to the genu of the corpus callosum (anterior part of rostral cingulate zone) and the posterior part of rostral cingulate zone. The present data support the notion that direct cortical innervation of the facial subnuclei from the CMAs might control upper face movement in humans, as previously implied in nonhuman primates. The CMAs may contribute to the sparing of upper facial muscles after a stroke involving the lateral precentral motor regions.

Keywords: bimanual movement, cingulate motor areas, face movement, motor control, neuroimaging

Introduction

A unilateral lesion involving the face representation of the primary motor cortex (M1), such as occurs after occlusion of the middle cerebral artery, typically causes contralateral lower facial palsy and largely spares upper facial movement. The traditional explanation for this discrepancy is that the facial subnuclei that control upper facial muscles receive bilateral innervation from M1; hence, the intact side alone can maintain the normal motor function of the upper face. Surprisingly, there is little experimental evidence to support this account. In nonhuman primates, the projection from M1 to the facial subnuclei governing the upper facial muscles is relatively weak in comparison with the lower facial muscles, albeit consistent (Jenny and Saper 1987; Morecraft et al. 2001). Rather, cingulate motor areas (CMAs) send a preferential projection to the dorsal and intermediate facial subnuclei controlling the orbicularis oculi and frontalis (Morecraft et al. 2001).

Previous human neuroimaging studies on blink movement have reported the involvement of both M1 and medial frontal motor areas (Bodis-Wollner et al. 1999; van Eimeren et al. 2001; Kato and Miyauchi 2003a; Yoon et al. 2005). However, those medial frontal activities have been ascribed to supplementary motor areas (SMAs), and the activation of CMAs has not been explicitly demonstrated thus far. SMAs in the medial frontal gyrus are often subdivided into the rostral part (SMAr or pre-SMA) and the caudal part (SMAc, SMA proper, or M2). SMAc/M2 is a somatotopically organized motor area (Fried et al. 1991; Luppino et al. 1991; Hanakawa et al. 2001). SMAc/M2 has been shown to project to the medial facial subnuclei that control the auricular musculature in nonhuman primates (Morecraft et al. 2001), but its role in facial expression is not yet clear in humans. CMAs are mostly buried within the cingulate sulcus (CS) and are also classified into several subregions representing somatotopy (Picard and Strick 1996). Anatomical evidence from nonhuman primates indicates that the rostral sector of CMA (M3) has a predominant role in controlling upper face movement (Morecraft et al. 2001). More recently, Gong et al. (2005) have performed a study on the cortical afferents to the motoneurons of orbicularis oculi, using a retrograde transneuronal tracer. They have shown premotor neurons of orbicularis oculi in both the M3 and the caudal sector (M4) of the CMAs. As it is suggested that humans possess the homologues of those somatotopically organized CMAs (Picard and Strick 1996), it is possible that CMAs participate in controlling upper face movement in humans as well. In agreement with this hypothesis, stimulation of the medial frontal regions including the cingulate areas with transcranial magnetic stimulation (TMS) can produce direct motor evoked potentials in the orbicularis oculi muscles (Sohn et al. 2004). In addition, a recent study on spontaneous blinking reported activity on the medial hemispheric wall at the border between medial Brodmann's area (BA) 6 (i.e., SMAs) and the cingulate areas (Yoon et al. 2005).

The anterior cingulate cortex is involved not only in simple motor execution but also in complex cognitive–motor behavior requiring response selection and conflict monitoring (Devinsky et al. 1995; Paus 2001). Clinical and neuroimaging evidence indicates that a part of the anterior cingulate cortex plays a critical role in the coordination of bimanual movement (Swinnen and Wenderoth 2004). However, none of the previous studies revealing cingulate activity with blinking have differentiated the activity from that commonly required for cued or bilateral movements. Therefore, to test the role of the CMAs in upper face movement in humans, we performed an event-related functional magnetic resonance imaging (fMRI) study in which brain activity was investigated during volitional closure of both eyes and bimanual tapping. Both types of movements were cued by the same visual stimulus. Bimanual tapping movement was employed to control for general task demands and to compare the location of activity with that of blinking-related activity on the medial frontal wall. It was hypothesized that the upper face representations of CMAs would be active during intentional blinking movement but not during bimanual movement.

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Materials and Methods

Subjects

Ten healthy right-handed subjects (6 women, 4 men), age 23–36, participated in the study. None had any previous history of neurological or psychiatric disorders. All subjects gave written informed consent approved by the institutional review board to participate.

Experimental Design

MRI scanning was conducted on a 3-Tesla scanner (GE, Milwaukee, WI). Subjects lay supine on the scanner bed with an individually molded bite bar to reduce head motion. Visual stimuli were back projected onto a screen, and subjects viewed them through a mirror built into a standard head coil.

During baseline periods, subjects fixated on a white crosshair in the center of a black rectangle. Physiological blinking was not suppressed throughout. To eliminate the cognitive effect of task selection, the task condition was fixed within an experimental run, either blinking or finger tapping. The temporal order of runs was pseudorandomized in each subject and was counterbalanced across subjects. The condition for each run was verbally instructed to the subject over an intercom system before the start of each run. During a run, the cue to perform the task was the disappearance of the white crosshair from the screen for 1.5 s, with an interstimulus interval of 21 s.

Blinking Task

For the blinking task, subjects quickly and completely closed their eyelids bilaterally in response to the visual cue. The blinking was a willful, voluntary movement that involved contraction of the orbicularis oculi muscles but not of the other body parts. The subjects were specifically instructed to avoid forceful blinks and not to move any other part of the face. One movement cycle (eyelid closing and opening) was performed per one visual cue.

Bimanual Tapping Task

For the tapping task, subjects quickly tapped the thumb to the index finger and then to the middle finger, using both hands simultaneously, in response to the visual cue.

Electromyography Recordings

In order to confirm the differentiated activation of the various muscle groups in each of the 2 tasks, 3 healthy subjects (age 23–38, 2 men and 1 woman) were studied separately by surface electromyography (sEMG) outside the MRI scanner. Silver–silver chloride adhesive disposable surface electrodes (Medtronic Inc, Minneapolis, MN) were placed to record from bilateral orbicularis oculi, frontalis, zygomaticus major, and first dorsal interossei (Cram et al. 1998). The subject was seated comfortably and performed visually cued bilateral blinking and bimanual tapping as above but with an interstimulus interval of 6 s. Finally, for this control experiment, sEMG was measured as the subjects performed repetitive smiling and forceful blinking to demonstrate the more widespread muscle activation that occurs in those tasks as compared with the experimental blinking task. sEMG electrode recordings were performed with a Neuropack EMG machine and MEB software (Nihon Kohden, Foothill Ranch, CA), with a sampling rate of 1 KHz, 10–500Hz band-pass filtering, 60 Hz notch filtering, and electrode placement as recommended by Cram (Cram et al. 1998).

MRI Acquisition

fMRI was based on the blood oxygenation level–dependent (BOLD) contrast. A transaxial gradient-echo, echo planar imaging sequence (GE/epiVP) was used with following parameters: repetition time = 2 s, echo time = 35 ms, field of view = 20 cm, matrix size = 64 × 64, 21 slices, voxel size = 3.1 × 3.1 × 6 mm. An experimental run consisted of 60 volumes, lasted 2 min, and included 5 cue events. Six experimental runs were obtained for each task (i.e., 30 events per task). A T_1-weighted 3-dimensional anatomical MRI was also obtained with a fast spoiled gradient recalled at steady-state sequence for each subject for anatomic coregistration.

Image Analysis

Image preprocessing and statistical analyses were performed using SPM99 (http://www.fil.ion.ucl.ac.uk/spm/) on Matlab (Mathworks, Inc., Natick, MA). Time series fMRI data were aligned in both time and space to account for differences in slice acquisition timing and head motion, respectively. The images were spatially normalized to fit to the Montreal Neurological Institute (MNI) template in a stereotaxic space and then were smoothed with a Gaussian filter of 10-mm full-width at half-maximum (FWHM). The final spatial resolution was ~15 mm.

Statistical analyses were conducted at both single-subject and group levels. The single-subject analysis was performed by correlating fMRI signal changes with gamma functions convolved with a canonical hemodynamic response function and its first-order time derivative (fixed effects model). A high-pass filter (90 s) and a low-pass filter (FWHM = 2 s) were applied. Parameter estimation was performed on the basis of the general linear model. The results from the single-subject analysis were assessed through t-statistics with planned linear contrasts on the parameter estimates. The single-subject analysis produced the parameter estimate images for each contrast for each subject. These "summary" images comprised the data for a second-stage analysis, treating subjects as a random variable (random effects model). One-sample t-tests were applied to the magnitude of event-related responses. In the second-level group analysis, we reported all activities that exceeded a threshold $P < 0.005$ and an extent threshold of $P < 0.05$ corrected for multiple comparisons. After the conversion of the MNI coordinates to the Talairach coordinates by a nonlinear transform (http://imaging.mrc-cbu.cam.ac.uk/imaging/MniTalairach), the activated regions were determined according to the Talairach Daemon Client (http://ric.uthscsa.edu/projects/talairachdaemon.html). When applicable, the SPM Anatomy Toolbox was used to estimate BA of activity.

For the direct comparison between the blinking and tapping activities, we also set a significance level at a height threshold of $P < 0.005$ and an extent threshold of $P < 0.05$ corrected for multiple comparisons. Nonnegligible activity observed at a height threshold of $P = 0.01$ was reported as a trend. For the blink-specific cingulate areas, where we had a preexisting hypothesis, we employed small volume correction (SVC) with a 7.5-mm radius spherical volume of interest (VOI) to test the significance of the activity. The coordinates (x, y, z = ±3, 12, 50) for the SVC analysis were taken from a previous study reporting a medial frontal activity during spontaneous blinking (Yoon et al. 2005). We employed this activity to seek cingulate blink areas because its peak was located around the border between BA6 and the cingulate areas. Signal time courses were computed for representative regions by setting 5-mm radius spherical VOIs centered at the voxels with the greatest task-selective difference in activity as determined by the second-level analysis. These VOIs were applied to the first-level time series data to extract BOLD signal time courses from the raw images. The data were converted to percent signal change for each trial and were then averaged across trials for each task for each subject.

As significant interindividual variation in the macroanatomy was reported in the anterior cingulate areas, the location of blinking-selective activity (blinking > tapping) was individually examined. The CS and its vertical branches were identified on each individual's anatomical MRI. The appearance of the paracingulate sulcus (PCS) was evaluated and classified into 3 categories (absent, present, and prominent) according to the criteria proposed by Paus et al. (1996). Statistical parametric maps of the blinking-minus-tapping contrast were computed from each individual and overlaid onto the subject's own T_1-weighted anatomical image for visual evaluation. In addition, blinking-selective activity in the precentral and medial wall of the frontal gyri was investigated in each individual in the same manner. For the individual-level analysis, the significance of activity was reported at 3 levels (see Table 3). 1) A threshold for significance was set at height threshold of $P < 0.05$ corrected for multiple comparisons (whole-brain correction) with an extent of at least 5 voxels. The statistical analysis at the individual level was more conservative in terms of the height threshold than at the group level because there was no intersubject anatomical variability. 2) An SVC method (7.5-mm radius VOI) was applied to the caudal part (coordinates: x, y, z = 2, 18, 46) and the rostral part (coordinates: x, y, z = 6, 32, 28) of the rostral cingulate zone and right precentral gyrus (coordinates: x, y, z = 48, 2, 48), where blink-specific activity was observed at the group level. The coordinates were based on the results from the group analysis. See Results and Table 2 for the details of each activity. 3) Nonsignificant activity observed at a height threshold of $P = 0.01$ was reported as a trend.

Results

Muscle Activity Patterns

Surface EMG revealed that during the blink task, orbicularis oculi were active bilaterally, mostly in isolation of frontalis and zygomaticus major (Fig. 1). However, one subject showed considerable EMG activity in the frontalis. The almost exclusive involvement of orbicularis oculi during the target-blinking task was in sharp contrast to the widespread facial muscle activity observed during the smiling task or the forceful blink task. The blinking task did not reveal any activity in the bilateral first dorsal interossei. On the other hand, the finger-tapping task was isolated to the first dorsal interossei and did not involve orbicularis oculi muscle activity.

Group Analysis of fMRI Data

General patterns of brain activity during the blinking and bimanual tapping tasks as compared with the fixation baseline are summarized in Table 1 and Figure 2. In brief, the bimanual tapping task produced brain activity in the bilateral M1, dorsal premotor cortex (PMd), primary somatosensory cortex, and medial frontal areas, in addition to the visual areas. The volitional blink task activated the medial frontal areas, bilateral opercular areas extending into the precentral gyrus on the right, and right temporoparietal areas.

When bimanual tapping-related activity was subtracted from blinking-related activity, there were no significant differences at the corrected threshold for the whole-brain volume. With the SVC analysis, however, blinking-specific activity was detected on the medial hemispheric wall at the level of the PCS (Paus et al. 1996) compatible with one of the CMAs (Table 2 and Fig. 3). The probability of this activity being BA6 was only 10% as reported by the SPM Anatomy Toolbox, and thus, it more likely belonged to the CMA than to the SMA. According to the stereotaxic coordinates, the blinking-selective CMA activity corresponded to the face representation of the posterior sector of rostral cingulate zone (RCZp) (Picard and Strick 1996). In addition, a trend toward blink-selective activity was found in a more rostral cingulate zone close to the genu of the corpus callosum ($x = 6$, $y = 32$, $z = 28$; z-score = 2.49) when a liberal threshold of uncorrected $P < 0.01$ was applied. This activity possibly corresponded to the anterior sector of the rostral cingulate zone (RCZa) (Picard and Strick 1996). A similar nonsignificant trend was noted in the right precentral area ($x = 48$, $y = 2$, $z = 48$; z-score = 2.78). A trend toward blinking-specific activity was also noted in the bilateral insular–opercular areas and visual association areas.

At the whole-brain corrected threshold, activity greater during tapping than blinking was observed in the bilateral perirolandic areas (M1, PMd, and primary somatosensory cortex), right parietotemporal area, and medial frontal areas including CMA and ventral SMA. The bimanual tapping-specific

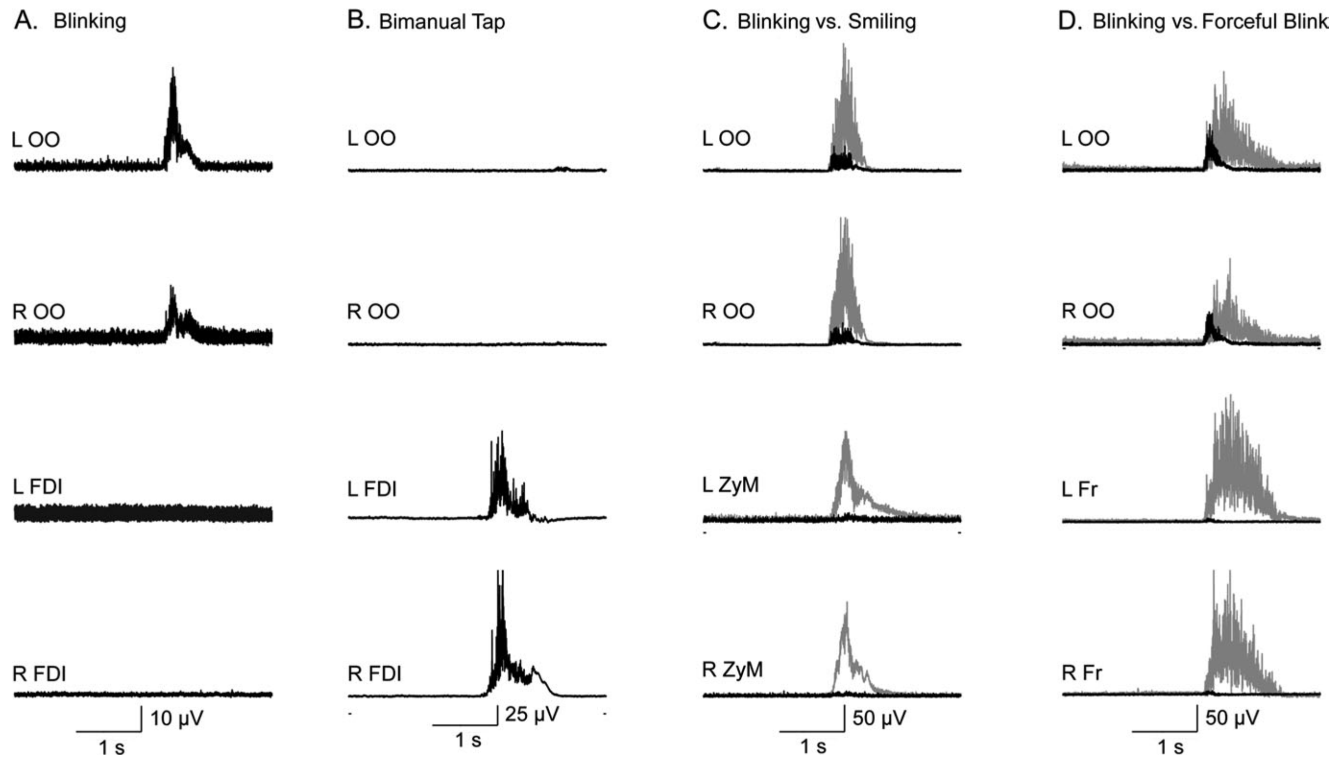

Figure 1. Muscle activity during the bilateral blinking and tapping tasks as measured by sEMG in a representative subject. (*A*) Muscle activity from the bilateral orbicularis oculi (OO) and first dorsal interossei (FDI) during the bilateral blinking task. sEMG was rectified and averaged over 10 trials. (*B*) Muscle activity from OO and FDI during the bimanual tapping task. (*C*) Muscle activity from the bilateral OO and zygomaticus major (ZyM) during the blinking (black) and smiling (gray) tasks. (*D*) Muscle activity from the bilateral OO and frontalis (Fr) during the blinking (black) and forceful blinking (gray) tasks. Note that almost no activity was recorded from the ZyM or Fr during the experimental blinking task in comparison with the smiling and forceful blinking tasks.

Table 1
Task-related activity as compared with the fixation baseline

Cluster	Corrected *P* value	Anatomic location (BA): functional areas	*z*-Score	Coordinates		
				x	*y*	*z*
A. Activity during the bimanual tapping task						
1	$P < 0.001$	L medial frontal gyrus (6): SMA	4.87	−4	−6	50
		R cingulate gurus (24): CMA	4.01	6	0	46
2	$P = 0.013$	R precuneus (7)	4.77	12	−80	44
		R cuneus (19)	3.70	2	−80	34
3	$P = 0.046$	R temporo-parietal junction (42): S2	4.45	62	−20	12
4	$P = 0.001$	L superior parietal lobule (7)	3.81	−24	−58	60
		L postcentral gyrus (1): S1	3.55	−54	−18	48
		L precentral gyrus (4): M1	3.54	−42	−22	52
5	$P < 0.001$	L lingual gyrus (18)	3.40	14	−76	0
6	$P < 0.001$	R middle frontal gyrus: PMd (6)	3.39	40	−6	54
		R post/precentral gyrus: S1/M1 (3/4)	3.32	40	−26	54
B. Activity during the blinking task						
1	$P < 0.001$	L/R cuneus (18)	5.13	0	−86	22
		L lingual gyrus (18)	4.93	−18	−72	−4
		R precuneus (7)	3.55	12	−78	48
2	$P < 0.001$	R cingulate gyrus (32): CMA	5.02	8	12	42
		R medial frontal gyrus (6): SMA	4.64	8	−4	64
		L/R cingulate gyrus (24): CMA	3.84	0	0	46
3	$P = 0.002$	L superior temporal gyrus (22)	4.66	−48	6	−6
		L inferior frontal gyrus (47)	4.16	−48	14	−4
4	$P = 0.010$	R superior temporal gyrus (13/22)	4.75	56	−46	16
		R inferior parietal lobule (40)	3.68	62	−38	40
5	$P < 0.001$	R inferior frontal gyrus (9)	4.11	56	12	28
		R precentral gyrus (6)	2.70	58	2	38

Note: S1, primary somatosensory area; the cluster numbers correspond to the labels in Figure 2. Activities are only listed that are considered significant with a height threshold of uncorrected $P < 0.005$ and an extent threshold of corrected $P < 0.05$. The listed stereotaxic coordinates are based on the MNI template, and the anatomic nomenclature is determined according to the Talairach Daemon Client after a nonlinear transformation to the Talairach coordinates.

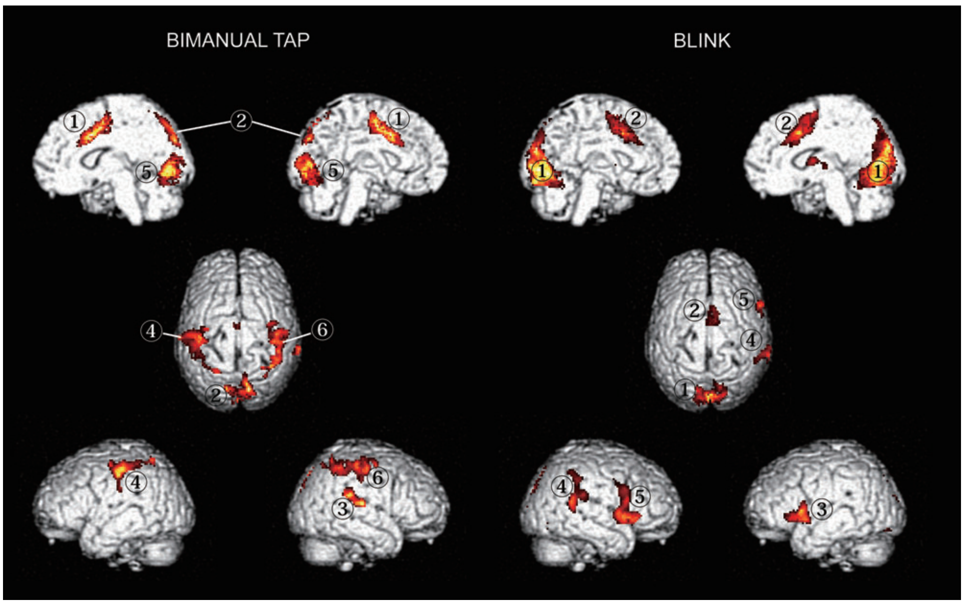

Figure 2. General patterns of brain activity during the bimanual tapping and blinking tasks compared with a fixation baseline in a second-level group analysis. The labels correspond to the cluster number in Table 1. The threshold is set at height threshold at uncorrected $P < 0.005$ and an extent threshold at $P < 0.05$ corrected for multiple comparisons.

medial activity corresponded to the arm representation of the caudal cingulate zone (CCZ) (Picard and Strick 1996). Event-related activity sampled from the blink-specific RCZp and the tapping-specific CCZ showed typical BOLD hemodynamic responses for blinking and tapping, respectively. Moreover, the plots illustrated the double dissociation of the task-specific signal time course across these 2 areas (Fig. 3).

Individual Analysis

As reported previously, considerable variability in gyrus–sulcus pattern was noted in the anterior cingulate areas (Table 3). Out of the 20 hemispheres from 10 subjects, the PCS was prominent in 7 hemispheres, present in 8 hemispheres, and absent in 5 hemispheres. Six subjects showed significant blinking-specific activity in the area consistent with RCZp (4 hemispheres with

Table 2
Direct comparison between the blink-related activity and the tapping-related activity

Corrected P value	Anatomic location (BA): functional areas	z-Score	Coordinates		
			x	y	z
Blink > tapping					
$P = 0.048$[a]	R CS (24): RCZp	2.81	2	18	46
$P = 0.23$	R inferior frontal gyrus (45)	3.58	58	16	4
$P = 0.43$	R middle occipital gyrus (19)	3.27	26	−56	2
$P = 0.48$	L inferior frontal gyrus (44)	3.22	−56	8	8
$P = 0.77$	L lingual gyrus (19)	2.87	−26	−68	−2
$P = 0.86$	R middle temporal gyrus	2.74	58	−48	10
$P = 0.83$	R precentral gyrus (4/6): M1	2.70	48	2	48
$P = 0.96$	R CS (24): RCZa	2.49	6	32	28
Tapping > blink					
$P = 0.014$[b]	L precentral gyrus (6): PMd	4.66	−32	−14	60
	L post/precentral gyrus (3/4): S1/M1	4.25	−42	−24	56
$P = 0.008$[b]	R postcentral gyrus (2): S1	4.37	52	−26	52
	R precentral gyrus (4): M1	4.26	38	−26	52
	R inferior parietal lobule (40)	4.18	42	−38	60
$P = 0.048$[b]	L CS (24): CCZ	4.12	−4	−6	44
	L medial frontal gyrus (6): SMA	3.11	−2	−14	54
$P = 0.07$	L caudate nucleus	3.96	−12	20	0
$P = 0.17$	R inferior temporal gyrus	3.64	56	−64	−2
$P = 0.43$	R superior temporal gyrus: S2	3.23	66	−26	14

Note: S2, second somatosensory areas.
[a]Significant ($P < 0.05$) after the SVC.
[b]Significant at a height threshold of uncorrected $P < 0.005$ and an extent threshold of corrected $P < 0.05$; other activities indicate a trend toward difference between the tasks ($P < 0.01$ uncorrected).

the whole-brain correction and 6 hemispheres with the SVC correction). Two more subjects showed a trend toward blink-specific RCZp activity (2 hemispheres). The mean stereotaxic coordinates were $x = 9.7$ mm, $y = 15.7$ mm, and $z = 48.0$ mm for the right hemisphere ($n = 7$) and $x = -4.0$ mm, $y = 20.4$ mm, and $z = 46.4$ mm for the left hemisphere ($n = 5$). Significant blink-specific activity was also found in the area corresponding to the RCZa in 5 subjects (8 hemispheres with the whole-brain correction). Three more subjects showed a trend toward blink-specific RCZa activity (4 hemispheres). The mean stereotaxic coordinates were $x = 11.0$ mm, $y = 27.5$ mm, and $z = 32.0$ mm for the right hemisphere ($n = 8$) and $x = -7.0$ mm, $y = 28.5$ mm, and $z = 32.0$ mm for the left hemisphere ($n = 4$). The relationship between the blinking-specific activity and the sulcus pattern was quite variable, mostly depending on the development of the PCS (Table 3 and Fig. 4). The blink-specific RCZp activity tended to be within the PCS or the vertical branch of the CS. The RCZa activity was more often observed within the CS (8 hemispheres) than the PCS (4 hemispheres).

Significant blinking-specific activity was observed in the right medial frontal gyrus in 3 subjects (3 hemispheres with the whole-brain correction), corresponding to the SMAc (see Subject 8 in Fig. 4). Another subject showed such a trend in the right medial frontal gyrus. The mean stereotaxic coordinates were $x = 11.0$ mm, $y = -4$ mm, and $z = 61.5$ mm ($n = 4$). Although blink-specific activity was not so robust in the lateral precentral areas at the group level, the individual analysis revealed blink-specific activity in the precentral gyri consistent with the M1 in 6 subjects (6 hemispheres with the whole-brain correction and 1 hemisphere with the SVC correction). Three more subjects showed such a trend. The mean stereotaxic coordinates were $x = 54.4$ mm, $y = 2.7$ mm, and $z = 39.6$ mm for the right hemisphere ($n = 9$) and $x = -45.7$ mm, $y = -0.7$ mm, and $z = 43.3$ mm for the left hemisphere ($n = 6$).

Discussion

Overall, the present blinking task yielded a brain activity pattern similar to that shown previously in the neuroimaging literature about intentional blinking (Bodis-Wollner et al. 1999; van Eimeren et al. 2001; Kato and Miyauchi 2003a) and natural blinking (Yoon et al. 2005). Also, the activation pattern during bimanual tapping was consistent with the previous reports (Stephan et al. 1999; Jancke et al. 2000; Immisch et al. 2001). In particular, the blinking and bimanual tapping tasks activated specific medial frontal structures as predicted. Blink-specific activity was found in the RCZp and RCZa representing face; to our knowledge, the present study was the first to demonstrate the involvement of these CMAs in upper face movement in humans. It should be noted that a very recent study found a cingulate activity during volitional suppression of blinking movement (Chung et al. 2006). CMA activity might have been overlooked in the previous studies as SMAs and CMAs are located nearby and often form an activity cluster at a group level when activated together. In our individual subject analysis, however, blink-specific activity was more consistently found in the RCAa or RCZp than in the SMAs. In addition, because previous imaging studies seldom compared blink-related activity statistically with other movement-related activity, it was possible that previously reported blink-related SMA activities could reflect general, nonsomatotopic motor initiation, or preparation. Another important issue is that the location of the upper face RCZa and RCZp was quite variable across the subjects in whom the development of the PCS substantially differed. The intersubject variability in location of the face RCZa and RCZp might have blurred blink-related activity there at a group level. Importantly, the present study is consistent with the evidence from the nonhuman primate study that emphasized the relative importance of the RCZa (M3 in monkeys) in controlling upper face movement (Morecraft et al. 2001). Further, the blinking-specific activity in the RCZp is in agreement with a recent demonstration of premotor neurons projecting to the orbicularis oculi in the M4 in monkeys (Gong et al. 2005). Based on the preliminary result of the present fMRI study, we performed a TMS experiment in which stimulation was directed at the medial part of the frontal lobe, including the CMAs (Sohn et al. 2004). This TMS experiment has demonstrated that medial frontal stimulation indeed elicits direct motor evoked potentials from the bilateral orbicularis oculi at 6–8 ms latency. Taken together with the present study, it seems likely that stimulation to CMAs rather than SMAs induced direct muscle activity from the orbicularis oculi.

The tapping-specific activity on the medial frontal wall was situated in the CCZ and SMAc, which has consistently been demonstrated during bimanual coordination tasks (Stephan et al. 1999; Jancke et al. 2000; Immisch et al. 2001). Both blinking and tapping tasks were fairly simple and unlikely to differ in difficulty. Other components such as recognition of the visual cues, attention to action, motor initiation, and behavioral monitoring should be the same between the 2 behavioral tasks. The component of the motor coordination of bilateral body parts was also intended to be controlled across the tasks, although coordination mechanisms might be somewhat different for different effectors. It is possible that there are special coordination systems for bimanual tapping which seems less naturally bilateral than blinking. In any event, the double

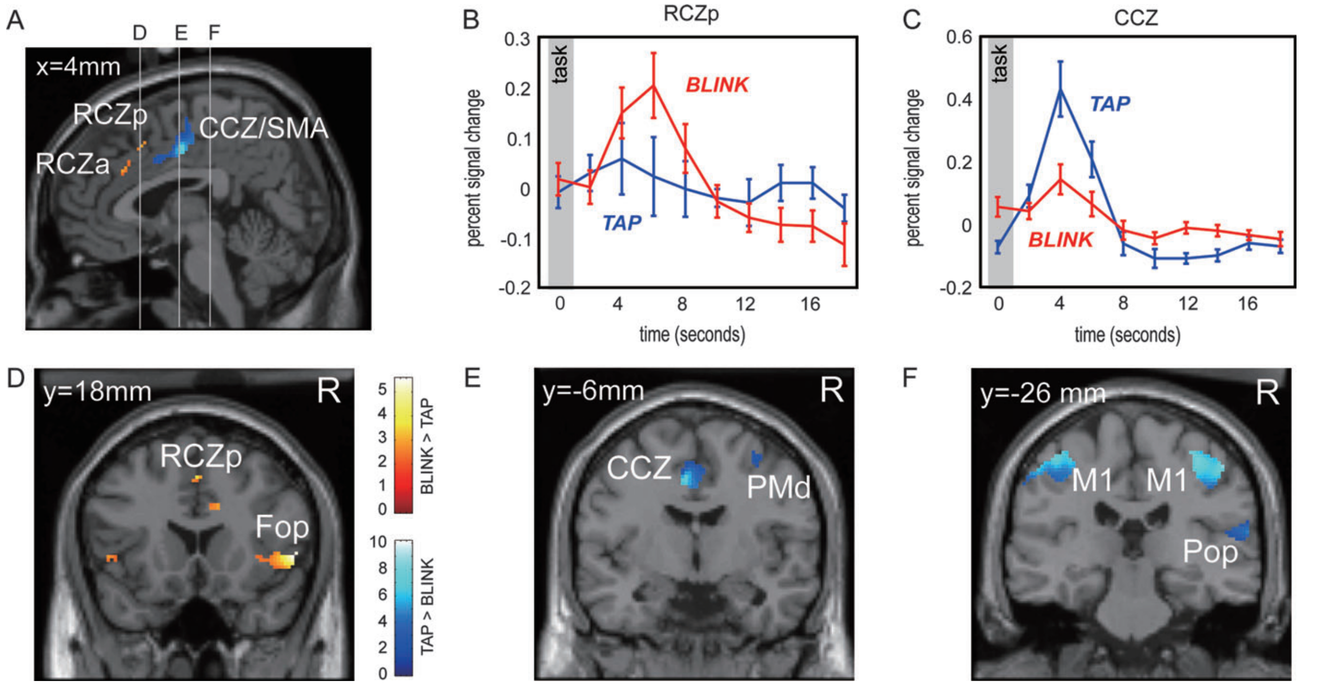

Figure 3. Blink- and tapping-specific activities from a second-level group analysis. Both activities are thresholded at uncorrected $P < 0.01$ for a display purpose. (*A*) Activities are superimposed on a sagittal plane of the standard MRI in the MNI space. The 2 CMA (RCZa and RCZp) showed activity greater during the blinking task than the bimanual tapping task. Conversely, the CCZ/SMA showed bimanual tapping-specific activity. The white vertical lines represent the coronal sections shown in (*D–F*). (*B*) A temporal profile of group-averaged tapping-specific BOLD signal changes in the RCZp. Time course activity was calculated from 5-mm spherical volumes of interest, was converted into a percent signal change, and was plotted against the time after the visual cue presentation (event onsets). The red line represents activity during the blinking task and the blue line indicates activity during the bimanual tapping task. Error bars indicate standard error of the mean. (*C*) A temporal profile of blinking-specific event-related activity in the CCZ. (*D–F*) The blink- and tapping-specific activities overlaid onto a coronal section of the standard MRI at $y = 18$ mm (*D*), $y = -6$ mm (*E*), and $y = -26$ mm (*F*). The blink-specific activity in the RCZp was significant after SVC, whereas the one in the frontal operculum (Fop) or RCZa did not reach the statistical significance. M1, hand representation of the primary motor cortex; Pop, parietal operculum.

Table 3

Individual analysis of blinking-specific activity (blinking > tapping) in the cingulate, medial frontal, and lateral precentral areas

	Sex	Side	PCS	RCZp					RCZa					SMA				M1			
				x	*y*	*z*	Sulcus	*z*-score	*x*	*y*	*z*	Sulcus	*z*-score	*x*	*y*	*z*	*z*-score	*x*	*y*	*z*	*z*-score
1	F	R	Pro	12	14	40	CS	3.20						10	−4	60	6.52[a]	58	−10	44	11.92[a]
		L	Pre															−48	0	46	3.29
2	M	R	Pre	2	2	50	CS	2.82	6	22	32	CS	2.36	14	−18	64	5.05[a]	46	10	40	3.50
		L	Abs																		
3	F	R	Abs						12	26	26	CS	2.44					62	−2	34	4.82[a]
		L	Pre	−8	20	52	CS–vbCS	4.32[b]										−48	−4	46	3.01
4	F	R	Pre	6	20	52	vbCS–PCS	3.04[b]	12	26	28	CS	5.03[a]					58	−2	34	4.31
		L	Pre						−10	32	26	CS	4.99[a]					−38	−2	40	3.68
5	F	R	Pro	8	32	50	PCS	4.86[a]	12	32	38	PCS	5.11[a]					56	12	36	2.42
		L	Pro	−2	22	50	PCS	3.38[b]	−4	34	40	PCS	3.25					−40	10	40	4.02
6	M	R	Pro	18	16	44	PCS	5.10[a]	12	36	32	PCS	5.99[a]					52	10	42	2.68[b]
		L	Pro	−4	18	46	PCS	2.78[b]													
7	F	R	Pre						6	28	38	PCS	2.67								
		L	Abs																		
8	M	R	Abs	12	10	48	CS	6.11[a]	14	24	34	CS	5.19[a]	8	−4	62	6.15[a]	54	−6	42	6.92[a]
		L	Pro	−2	20	40	vbCS	3.03[b]	−8	22	28	CS	5.39[a]					−52	−6	42	3.09
9	F	R	Pre	6	18	52	vbCS–PCS	3.29[b]	14	26	28	CS	5.44[a]	12	10	60	2.75	46	4	50	5.08[a]
		L	Pro	−4	22	44	vbCS	3.99[a]	−6	26	34	CS	4.69[a]								
10	M	R	Pre															58	8	34	5.39[a]
		L	Abs															−48	−2	46	5.98[a]

Note: Abs, absent; Pre, present; Pro, prominent; vbCS, vertical branch of cinglulate sulcus.
[a]Significant at a height threshold corrected for multiple comparisons ($P < 0.05$; more than 5 voxels).
[b]Significant at the SVC corrected threshold; other activities indicate a trend toward difference between the tasks ($P < 0.01$ uncorrected).

dissociation of the blinking- and tapping-related activities strongly indicates that the representations of the bilateral blinking and bimanual tapping are segregated in the cingulate/medial frontal areas. Moreover, the individual analysis clearly showed the 2 distinct upper face representations in the cingulate areas. These results support the somatotopically organized multiple CMA scheme proposed by Picard and Strick (1996).

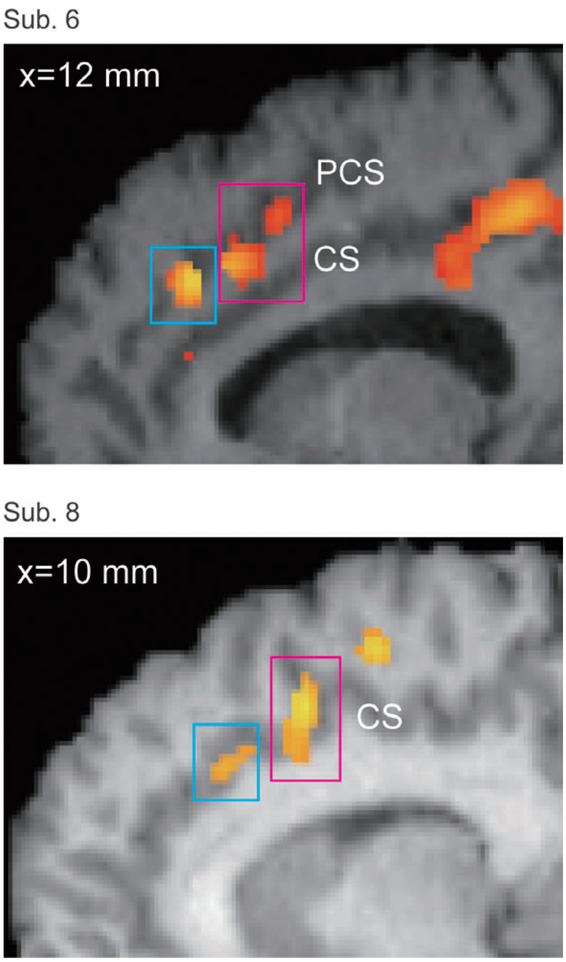

Figure 4. The blinking-specific activity in the CS or the PCS. Individual analyses were performed to examine the relationship between the blinking-specific (blinking > tapping) activity and the sulcus pattern of the cingulate areas (see also Table 3). The prominent PCS in the right hemisphere of Subject 6 contained blinking-specific activity for both the RCZa (cyan) and RCZp (magenta). The PCS was absent in the right hemisphere of Subject 8, and the blinking-specific activity was within the cingulate sulucus for both the (cyan) and RCZp (magenta). Note also that this particular subject had blink-specific activity in the medial frontal gyrus consistent with SMA. However, blink-specific SMA activity was not very consistent across subjects.

During the blinking task, involvement of other facial muscles, especially that of the frontalis, cannot be completely excluded. However, the sEMG recording showed the predominant involvement of the bilateral orbicularis oculi, largely in isolation from other facial muscles under the condition of the blinking task employed here. Another issue in the interpretation is the involvement of undesigned eye movement. Previous neuroimaging studies have used oculomotor movements that have close relationships with blinking movements as their control tasks and found overlapping brain activity between the 2 tasks (Bodis-Wollner et al. 1999; Kato and Miyauchi 2003b). Physiological coupling of blinking with eye movement may largely explain the overlap of oculomotor- and blink-related activities. In the present study, as oculomotor tasks were not included as a control condition, we could not exclude the possibility that the present CMA activities reflected oculomotor components associated with blinking rather than blinking movement itself. In a follow-up fMRI experiment, therefore, brain activity during upper face movement without involving eye movement (raising eyebrows) was compared with activity during vertical eye movement. A preliminary analysis has shown that activity during the upper face movement is overlapped with activity during the vertical eye movement in both cingulate and medial frontal areas; however, significant activity remains, after subtracting the eye movement–related activity from the forehead movement–related activity (Hanakawa and Fukuyama 2005). This preliminary finding suggests that the upper face and oculomotor representations are adjacent but are segregated in the cingulate/medial frontal motor areas and is compatible with the interpretation that the present blink-specific activity included the upper face representations of the CMAs.

Most previous imaging studies observed blink-related activity in the lateral precentral gyri (Bodis-Wollner et al. 1999; van Eimeren et al. 2001; Kato and Miyauchi 2003b). In the present study, brain activity was significantly greater during blinking movement than the baseline in the opercular area, and this lateral frontal activity extended into the precentral gyrus in the right hemisphere. Further, the blink-specific precentral activity was clearly observed at the individual level. The stereotaxic coordinates of the blink-specific precentral activity (mean z-coordinate = 40–43 mm) are almost the same with or slightly dorsal to those of the lower face movement–related precentral activity in our previous fMRI study (mean z-coordinate = 36–38 mm) (Hanakawa et al. 2005). This spatial relationship of the upper and lower face motor representations agrees with the somatotopic organization of the precentral motor areas. It is thus likely that upper face movement in humans is subserved by multiple cortical motor regions rather than exclusively by the CMAs. In fact, Gong et al. (2005) found that, in addition to CMAs, multiple motor areas including the M1 and lateral premotor areas contain premotor neurons of the orbicularis oculi. The lateral precentral areas project predominantly to the contralateral lower facial muscles and relatively weakly, but consistently, to the upper facial musculature via the facial nucleus (Morecraft et al. 2001). This projection accounts for the movement patterns elicited by cortical stimulation to the lateral cerebral hemisphere (Cohen and Hallett 1988; Paradiso et al. 2005). At the group level, blink-related precentral activity was bilateral but was more pronounced in the right hemisphere. Notably, the blinking-specific CMA activity was found more consistently on the right side, which may together provide supporting evidence about the predominant role of the right hemisphere in controlling volitional blinking (van Eimeren et al. 2001). The precentral zone might be strongly related to the volitional aspect of the blinking movement because natural blinking shows medial frontal activity but lacks precentral activity (Yoon et al. 2005). The dual innervation of the facial musculature by the lateral and medial motor areas could be related to the fact that there are 2 principal varieties of central facial palsy: the volitional and emotional types.

Our series of imaging and TMS studies have provided supportive evidence for a role of CMAs in controlling upper face movement in humans. The cortical innervation of the facial subnuclei from the CMAs may contribute to the sparing of upper facial muscles after a stroke involving the lateral precentral gyrus in humans. The relative contribution from the medial and lateral motor areas to the control of upper facial movement should be further investigated in the context of volitional and emotional control of facial expression (Hopf et al. 1992; Wild et al. 2003). This issue may also have implication for disorders involving blinking such as blepharospasm and motor tic disorders (Devinsky et al. 1995). Involuntary forceful blinking is one of the most frequent symptoms in tic disorders. In the meantime, it would be worthwhile investigating upper facial weakness carefully in patients with medial frontal lesions to test the role of medial frontal motor areas in the control of the upper facial musculature.

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Notes

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References

Bodis-Wollner I, Bucher SF, Seelos KC. 1999. Cortical activation patterns during voluntary blinks and voluntary saccades. Neurology. 53:1800–1805.

Chung JY, Yoon HW, Song MS, Park H. 2006. Event related fMRI studies of voluntary and inhibited eye blinking using a time marker of EOG. Neurosci Lett. 395:196–200.

Cohen LG, Hallett M. 1988. Noninvasive mapping of human motor cortex. Neurology. 38:904–909.

Cram JR, Kasman GS, Holtz J. 1998. Introduction to surface electromyography Gaithersburg (MD): Aspen Publishers, Inc.

Devinsky O, Morrell MJ, Vogt BA. 1995. Contribution of anterior cingulate cortex to behaviour. Brain. 118:279–306. [Review].

Fried I, Katz A, McCarthy G, Sass KJ, Williamson P, Spencer SS, Spencer DD. 1991. Functional organization of human supplementary motor cortex studied by electrical stimulation. J Neurosci. 11: 3656–3666.

Gong S, DeCuypere M, Zhao Y, LeDoux MS. 2005. Cerebral cortical control of orbicularis oculi motoneurons. Brain Res. 1047: 177–193.

Hanakawa T, Fukuyama H. 2005. Cortical control of upper facial movement: an fMRI study. Program No. 288.14. 2005 Abstract Viewer/Itinerary Planner. Washington, DC: Society for Neuroscience, 2005. Online.

Hanakawa T, Ikeda A, Sadato N, Okada T, Fukuyama H, Nagamine T, Honda M, Sawamoto N, Yazawa S, Kunieda T, et al. 2001. Functional mapping of human medial frontal motor areas. The combined use of functional magnetic resonance imaging and cortical stimulation. Exp Brain Res. 138:403–409.

Hanakawa T, Parikh S, Bruno MK, Hallett M. 2005. Finger and face representations in the ipsilateral precentral motor areas in humans. J Neurophysiol. 93:2950–2958.

Hopf HC, Muller-Forell W, Hopf NJ. 1992. Localization of emotional and volitional facial paresis. Neurology. 42:1918–1923.

Immisch I, Waldvogel D, van Gelderen P, Hallett M. 2001. The role of the medial wall and its anatomical variations for bimanual antiphase and in-phase movements. Neuroimage. 14:674–684.

Jancke L, Peters M, Himmelbach M, Nosselt T, Shah J, Steinmetz H. 2000. fMRI study of bimanual coordination. Neuropsychologia. 38: 164–174.

Jenny AB, Saper CB. 1987. Organization of the facial nucleus and corticofacial projection in the monkey: a reconsideration of the upper motor neuron facial palsy. Neurology. 37:930–939.

Kato M, Miyauchi S. 2003a. Functional MRI of brain activation evoked by intentional eye blinking. Neuroimage. 18:749–759.

Kato M, Miyauchi S. 2003b. Human precentral cortical activation patterns during saccade tasks: an fMRI comparison with activation during intentional eyeblink tasks. Neuroimage. 19:1260–1272.

Luppino G, Matelli M, Camarda RM, Gallese V, Rizzolatti G. 1991. Multiple representations of body movements in mesial area 6 and the adjacent cingulate cortex: an intracortical microstimulation study in the macaque monkey. J Comp Neurol. 311:463–482.

Morecraft RJ, Louie JL, Herrick JL, Stilwell-Morecraft KS. 2001. Cortical innervation of the facial nucleus in the non-human primate: a new interpretation of the effects of stroke and related subtotal brain trauma on the muscles of facial expression. Brain. 124:176–208.

Paradiso GO, Cunic DI, Gunraj CA, Chen R. 2005. Representation of facial muscles in human motor cortex. J Physiol. 567:323–336.

Paus T. 2001. Primate anterior cingulate cortex: where motor control, drive and cognition interface. Nat Rev Neurosci. 2:417–424.

Paus T, Tomaiuolo F, Otaky N, MacDonald D, Petrides M, Atlas J, Morris R, Evans AC. 1996. Human cingulate and paracingulate sulci: pattern, variability, asymmetry, and probabilistic map. Cereb Cortex. 6: 207–214.

Picard N, Strick PL. 1996. Motor areas of the medial wall: a review of their location and functional activation. Cereb Cortex. 6:342–353.

Sohn YH, Voller B, Dimyan M, St Clair Gibson A, Hanakawa T, Leon-Sarmiento FE, Jung HY, Hallett M. 2004. Cortical control of voluntary blinking: a transcranial magnetic stimulation study. Clin Neurophysiol. 115:341–347.

Stephan KM, Binkofski F, Halsband U, Dohle C, Wunderlich G, Schnitzler A, Tass P, Posse S, Herzog H, Sturm V, et al. 1999. The role of ventral medial wall motor areas in bimanual co-ordination. A combined lesion and activation study. Brain. 122 (Pt 2):351–368.

Swinnen SP, Wenderoth N. 2004. Two hands, one brain: cognitive neuroscience of bimanual skill. Trends Cogn Sci. 8:18–25.

van Eimeren T, Boecker H, Konkiewitz EC, Schwaiger M, Conrad B, Ceballos-Baumann AO. 2001. Right lateralized motor cortex activation during volitional blinking. Ann Neurol. 49:813–816.

Wild B, Rodden FA, Grodd W, Ruch W. 2003. Neural correlates of laughter and humour. Brain. 126:2121–2138.

Yoon HW, Chung JY, Song MS, Park H. 2005. Neural correlates of eye blinking; improved by simultaneous fMRI and EOG measurement. Neurosci Lett. 381:26–30.