Neural substrates of the warning effect: a functional MRI study

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Contents

Abstract

Motor response reaction times towards a target decrease when presentation is preceded by a stimulus indicating that the target will appear shortly. I used functional magnetic resonance imaging to depict the neural substrates of this warning effect during a Go/NoGo task incorporating a warning stimulus. I hypothesized that the warning stimuli activate the midbrain-thalamic-ACC alertness system irrespective of stimulus modality, and the areas which are related to early stage of motor processing such as pre-supplementary motor area (pre-SMA). Fifteen subjects completed a visual Go/NoGo task, and 12 completed an analogous task in the auditory modality. After a variable-duration warning stimulus was presented, a Go or NoGo cue was presented with equal probability. When a Go cue was presented, subjects had to respond as rapidly as possible by pressing a button. When a NoGo cue was presented, subjects were required not to respond. Auditory and visual warning stimuli commonly activated the midbrain and thalamus, which are associated with tasks involving directed attention and alertness. Warning-related activation was found in the anterior cingulate cortex (ACC), which might monitor the task-related utility of the warning stimulus, and in the

pre-supplementary motor area (pre-SMA), which is associated with movement selection. Warning stimuli might potentiate pre-SMA activity related to movement selection and/or preparation, through the midbrain–thalamus–ACC alerting system.

1. Introduction

The presence of a cueing stimulus before a target (warning stimulus) reduces reaction times (RTs) when individuals make motor responses towards the target. This reduction in RT is called as warning effect. This effect is seen even if the warning stimulus conveys no information about the nature of the imperative stimulus or the required responses (Hackley and Valle-Inclán, 2003). The ability to increase and maintain response readiness in preparation for an impending stimulus is called alertness, and is regarded as a fundamental form of attention (Raz and Buhle, 2006). Alertness can be broadly subdivided into two types, phasic alertness and intrinsic alertness, the latter of which can also be called vigilance, arousal, or sustained attention (Sturm et al., 1999; Sturm and Willmes, 2001; Raz and Buhle, 2006). In general, a behavioral measure of tonic or intrinsic alertness is provided by the RT in trials lacking any warning stimulus, while the reduction in RT produced by prior presentation of a warning stimulus is thought to be a measure of phasic alertness (Sturm et al., 1999; Coull et al., 2001). Thus, the warning effect is considered to be a result of increased phasic alertness triggered by the warning stimuli.

Previous imaging studies have suggested that the phasic and intrinsic alerting networks include the midbrain–thalamic–anterior cingulate cortex (ACC) system (Kinomura et al., 1996; Sturm et al., 1999; Sturm and Willmes, 2001; Fan et al., 2005; Raz and Buhle, 2006). Kinomura et al. (1996) conducted a positron emission tomography (PET) study with an attention-demanding RT task using either visual or somatosensory cues. They found that, irrespective of sensory modality, the midbrain reticular formation and thalamic intralaminar nuclei were activated when participants moved from a relaxed and awake state into attention-demanding task trials. Using PET with O-15 water, Paus et al. (1997) showed that thalamic blood-flow responses to an auditory vigilance task covaried with those of the ponto-mesencephalic tegmentum and the ACC. During an auditory vigilance study, blood-flow responses in the ACC, thalamus, and mesencephalic reticular formation decreased at similar rates over a 50-min testing period; these changes occurred together with increases in the response latency and in the amount of electroencephalographic activity in the theta frequency range. These observations suggest that activation of the ACC is modulated by the arousal state of the organism, and is therefore likely to be associated with tonic

alertness.

The ACC is located in the medial wall of the cerebral hemisphere. The ventral (limbic) tier occupies the surface of the cingulate gyrus, corresponding to Brodmann's areas 24a and 24b, and the subcallosal area 25. The dorsal (paralimbic) tier is buried in the cingulate sulcus corresponding to Brodmann's areas 24c and 32 (see Paus, 2001, for a review). The ACC receives dense inputs from the midline thalamic nuclei (Barbas and Pandya, 1989), which are involved in the regulation of cortical arousal (Montaron and Buser, 1988). Arousal-related modulation of the ACC might also arise from brainstem monoamine nuclei inputs, such as those from the mesocortical dopamine system originating in the ventral tegmental area, and noradrenaline inputs from the locus coeruleus (LC) (Barger, 1992; Crino et al., 1993). The cingulate motor areas (CMAs) in the cingulate sulcus receive inputs from the primary motor cortex (M1), premotor cortex, and supplementary motor area (SMA), and give rise to cortical spinal projections (Dum and Strick, 1991; Morecraft and Van Hoesen, 1992). The ACC also has dense connections with prefrontal cortical areas (PFCs) (Barbas and Pandya, 1989), and the CMA also receives PFC inputs (Bates and Goldman-Rakic, 1993; Morecraft and Van

Hoesen, 1993; Picard and Strick, 1996). These anatomical connections suggest that the connectivity of the ACC has three important features relating to behavioral control (Paus, 2001). First, extensive afferents from the thalamus and the brainstem nuclei suggest the involvement of the ACC in arousal control (Paus, 2001). Second, dense projections from the ACC to the motor cortex and the spinal cord indicate a role for the ACC in motor control. Third, reciprocal cortico–cortical connections between the ACC and the lateral PFC might represent a mechanism for communication between cognitive and motor systems. Given this anatomical evidence for the convergence of cognitive and motor processes, as well as inputs relaying information about the alertness of the organism, I hypothesize that the ACC is a key neural substrate for the warning effect. This is consistent with the concept of an anterior alerting system proposed by Sturm and Willmes (2001).

While previous imaging studies have suggested that tonic alerting networks include the midbrain-thalamic-ACC system irrespective of stimulus modality (Kinomura et al., 1996), Fan et al. (2005) showed that the phasic alerting network also includes the midbrain-thalamic-ACC system using warning stimuli. But they used only

visual task, it was unclear whether this system is active irrespective of modality. Thus, it's unknown whether the midbrain-thalamic-ACC system is active irrespective of stimulus modality in not only tonic but also phasic alertness.

Although the warning effect is a well-replicated behavioral observation, it is unclear which aspects of cognitive or motor processing are facilitated by the presentation of a warning stimulus. Previous studies of event-related potentials suggest that the reduction of RT associated with the warning effect is caused neither by the facilitation of low-level motor processes nor by sensory-perceptual processing, but rather by nonspecific motor "priming" within an early phase of response selection (Hackley and Valle-Inclán, 2003). This raises the possibility that the warning effect is mediated by the midbrain–thalamus–ACC network, which is thought to facilitate movement preparation in the premotor cortices, irrespective of stimulus modality. I conducted a functional magnetic resonance imaging (fMRI) study to test the hypothesis that warning stimuli activate the midbrain-thalamic-ACC alertness system irrespective of stimulus modality, and the areas which are related to early stage of motor processing such as pre-supplementary motor area (pre-SMA). I adopted a visual and auditory

Go/NoGo task with warning stimuli that had warning periods of variable duration. This paradigm allowed me to differentiate the responses to the warning stimuli, as well as the Go and NoGo cues. The application of both visual and auditory tasks allowed me to investigate modality-invariant effects, and the variable warning period allowed me to exclude factors related to temporal orienting, which allowed specific predictions to be made about the timing of the presentation of Go or NoGo cues (Coull et al., 2001). Specifically, I expected the warning stimuli to activate both the alerting system and those areas that are involved in the early stages of motor processing, such as movement preparation.

2. Materials and Methods

2.1. Participants

In total, 51 right-handed healthy volunteers took part in the study. Twenty-four subjects participated in the preliminary psychological testing (12 males and 12 females; mean age, 27.9 ± 4.8 years). Twenty-seven healthy right-handed volunteers participated in the fMRI study. Fifteen of these (seven males and eight females; mean age, 24.1 ± 2.3 years) completed a visual Go/NoGo task, and the remaining 12 (seven males and five females; mean age, 22.8 ± 3.4 years) completed an auditory version of the Go/NoGo task. All subjects were right-handed according to the Edinburgh handedness inventory (Oldfield, 1971). None of the subjects had a history of psychiatric or neurological illness. The protocol was approved by the ethical committee of the National Institute for Physiological Sciences, Japan, and all subjects gave their written informed consent to participate.

2.2 Tasks and preliminary psychological testing

I used Go/NoGo tasks with either auditory or visual stimuli. In the preliminary

psychological testing, subjects completed four versions of the Go/NoGo task, differing in stimulus modality (visual or auditory) and the presence or absence of a warning stimulus as follows: visual warning (VW), visual no-warning (VN), auditory warning (AW), and auditory no-warning (AN) tasks. This was intended to establish the fact that the VW and AW task had a warning effect, as measured by a reduction in the RT. In the fMRI protocol, 15 and 12 subjects completed the VW and AW tasks, respectively.

In the VW task (Figure 1a), a trial began with the presentation of a central fixation point (a white cross). The subjects were asked to fixate on the position of this white cross during the session. Following this, the central fixation point turned yellow (the warning period). After a warning period of variable duration (2–6 s), the warning stimulus was replaced by either a blue square (Go cue) or a red square (NoGo cue) presented in the center of the display. The duration of the Go and NoGo cues was 350 ms. When a Go cue was presented, the subjects had to push a button with their right thumb as quickly as possible. When a NoGo cue was presented, the subjects were required not to push the button. Each visual stimulus was presented at a visual angle of 1.3° × 1.3°. After the cue disappeared, the white cross was presented again, with an

inter-trial interval (ITI) of 12–14 s, until the beginning of the next warning period. The VW task consisted of two successive experimental sessions, which together comprised 10 Go trials and 10 NoGo trials. The trial order, the ITI, and the duration of the warning period were pseudorandomized across the two sessions. The VN task was identical to the VW task except for the absence of warning stimuli. The AW task was identical to the VW task except for the task-related stimulus modality and the presentation of the white cross, which was displayed throughout the session. In the AW task, the warning stimuli consisted of a pure-tone stimulus (frequency, 440 Hz; sampling rate, 44.1 kHz; stereo sound) presented for 2–6 s. A higher-pitched pure tone (frequency, 480 Hz; sampling rate, 44.1 kHz; stereo sound) was used as the Go cue, and a lower-pitched pure tone (frequency, 400 Hz; sampling rate, 44.1 kHz; stereo sound) was used as the NoGo cue. The AN task was identical to the AW task except for the absence of warning stimuli. Each subject completed two sessions of each task type, the order of which was counterbalanced across all subjects, giving a total of eight sessions, including 40 Go and 40 NoGo trials.

In the preliminary psychological testing, the stimulus presentation and response

collection were controlled by Presentation software (Neurobehavioral Systems, Albany, CA, USA) on a personal computer (Dimension 9100; Dell Computer Co., Round Rock, Texas, USA). The visual stimuli were presented on a 19-inch liquid-crystal display (Diamondcrysta RDT197V; Mitsubishi Electric, Tokyo, Japan). The auditory stimuli were presented via a stereo-speaker system (SRS-A202; Sony, Tokyo, Japan). The responses were collected via an in-house button system that was made from a universal serial bus (USB)-connected numeric keypad (TNK-SUU211GY; LOAS Co., Ltd, Osaka, Japan) and a switch (Z-15GD; Omuron, Kyoto, Japan).

For each subject, the accuracy and the mean RT for the correct responses were calculated. Responses with a delay of longer than 1 s were regarded as missed responses. Statistical analysis was carried out using SPSS version 10.0J software (SPSS Japan Inc., Tokyo, Japan). A two-way repeated measure analysis of variance (ANOVA) was performed to compare the mean percentage of correct responses and the mean RT. The results were considered statistically significant at $p < 0.05$.

2.3. fMRI study

Prior to being scanned, each subject completed behavioral training. The training consisted of six tasks: three RT tasks and three Go/NoGo tasks. The time for response was gradually decreased until the performance reached a certain criterion (determined by RTs of each individual) level. After the training, the subjects performed a visual or auditory Go/NoGo task (Figure 1a), which was similar to the VW and AW tasks, respectively, used for the preliminary psychological testing inside the scanner, differing only in the visual angle and the number of sessions. In the visual task (Figure 1a), each stimulus was presented at a visual angle of 1.9° × 1.9°, and the two successive sessions were repeated four times giving a total of eight sessions, including 40 Go trials and 40 NoGo trials. The accuracy and RTs in response to the Go cues were analyzed in the same way as the preliminary psychological data (described above).

For both stimulus modalities, target stimuli in the Go/NoGo tasks were counterbalanced across subjects to control for possible stimulus-specific effects: in the visual task, the red square was used as the Go cue and the blue square was used as the NoGo cue for half of the participants, while this combination was reversed for the

remaining half of the participants. Similar counterbalancing was applied to the high-pitch and low-pitch tones used as cues in the auditory tasks.

Presentation 9.81 (Neurobehavioral Systems, Albany, CA) was implemented on a personal computer (Dimension 9100; Dell Computer, Round Rock, TX) for the stimulus presentation and response collection. A liquid-crystal display projector (DLA-M200L; Victor, Yokohama, Japan), located outside and behind the scanner, projected stimuli through another waveguide onto a translucent screen, which the subjects viewed via a mirror attached to the MRI head coil. The auditory stimuli were presented via MRI-compatible headphones (Hitachi, Yokohama, Japan). The volume was adjusted to approximately 90 dB. Responses were collected via an optical button system (Current Design, Philadelphia, PA).

2.4. MRI data acquisition

All images were acquired using a 3T MR scanner (Allegra; Siemens, Erlangen, Germany). Functional images were acquired using a T2*-weighted echo-planar imaging (EPI) sequence. Each volume consisted of 34 slices, each of which was 4.0 mm thick,

with a gapless alignment to cover the entire cerebrum and cerebellum (repetition time [TR], 2,000 ms; echo time [TE], 30 ms; flip angle [FA], 80°; field of view [FOV], 192 mm; 64 × 64 matrix; voxel dimensions, 3 × 3 × 4 mm). Oblique scanning was used to exclude the eyeballs from the images. The onset of each trial, relative to the preceding image acquisition, was jittered within 1 TR (2,000 ms) (Dale, 1999). For anatomical imaging, T1-weighted magnetization-prepared rapid-acquisition gradient-echo (MP-RAGE) images, scanned at the same locations as those used for the EPI, were obtained for each subject (TR, 1,460 ms; TE, 4.38 ms; FA, 8°; FOV, 192 mm; matrix size, 256 × 256 mm; voxel dimensions, 0.9 × 0.8 × 4.0 mm). To acquire a fine structural whole-head image, MP-RAGE images were also obtained (TR, 2,500 ms; TE, 4.38 ms; FA, 8°; FOV, 230 mm; matrix size, 256 × 256 mm; voxel dimensions, 0.9 × 0.9 × 1.0 mm).

Each session consisted of a continuous series of 112 volumes acquired with a total duration of 3 min 44 s. To avoid subject fatigue, several breaks (of up to 5 min) were inserted between the eight sessions. The total duration of the experiment was approximately 70 min, including the acquisition of the structural MR images.

2.5. fMRI data analysis

In addition to the first two volumes of each EPI sequence, which were discarded automatically by the MR scanner, the first three volumes of each fMRI session were removed from the analysis to allow for stabilization of the magnetization, and the remaining 109 volumes per session (a total of 872 volumes per subject for eight sessions) were used in the analysis. The data were analyzed using statistical parametric mapping (SPM5; Wellcome Department of Imaging Neuroscience, London, UK) implemented in Matlab (Mathworks, Sherborn, MA) (Friston et al., 1994; Friston et al., 1995; Friston et al., 2007). After correcting for differences in slice timing within each image volume, all volumes were realigned for motion correction. All EPI volumes were normalized to the Montréal Neurological Institute (MNI) EPI template using a nonlinear basis function. The functional images were spatially smoothed in three dimensions using an 8-mm full-width at half-maximum Gaussian kernel.

Statistical analysis of functional data was conducted at two levels. First, individual task-related activation was evaluated. Second, the summary data for each

individual were incorporated into the second-level analysis using a random-effect model (Friston et al., 1999) to make inferences at the group level.

The each EPI image was scaled proportionally by setting the mean value of whole brain of all volumes to 100 arbitrary units. The signal time-course for each subject was modeled using a hemodynamic response function, session effect, and high-pass filtering (128 s). To test my hypotheses about regionally-specific condition effects, the model parameters were compared with the linear contrasts. I used an event-related design that consisted of the following three types of event condition: Warning, Go, and NoGo (Figure 1b). The design matrix (X) was created by convolving a set of three vectors with a hemodynamic response function (h) as follows,

$$X = [w, g, n] \otimes h,$$

where w corresponds to Warning, g corresponds to Go, and n corresponds to NoGo. I excluded the incorrect-Go and NoGo trials for further analysis. Initially, I delineated the areas that were related to the onset of the warning period. A Go–NoGo comparison was conducted to depict the neural substrates associated with the motor execution of hand movement.

The weighted sum of the parameter estimates in the individual analysis constituted "contrast" images, which were used for the group analysis (Friston et al., 1999). Contrast images obtained via individual analysis represented the normalized task-related increment of the MR signal of each subject. The contrast images of Warning, Go, and NoGo for each cueing modality were incorporated into a flexible factorial design that modeled the subject effect, cueing modality effect, and conditions (Warning, Go, and NoGo) at the group level. The resulting set of voxel values for each contrast constituted a statistical parametric map of the *t* statistic (SPM{*t*}). I delineated the activation of the warning effect irrespective of the cue modality by masking with the contrast of both visual and auditory warning at a statistical threshold of $p < 0.05$, family-wise error (FWE) corrected at the voxel level (Friston et al., 1996), with the cluster size set at >20 voxels. Similarly, the execution effect as observed in the Go–NoGo comparison irrespective of the cue modality was evaluated.

Two additional analyses were performed to confirm the warning effect. First, parametric analysis of warning condition was performed by using partial correlation analysis that was implemented in SPM5 (Buchel et al., 1998). Data analysis was

performed by modeling individual subject's reaction time of correct Go trials as delta functions convolved with a canonical hemodynamic response function of warning condition prior to the Go trial. Thus, individual subject's reaction time of correct Go trials was entered as warning related regressors in design matrix. By using this design matrix, I evaluated the areas that indicate the positive and negative correlation with reaction time when the warning stimulus was presented. The contrast images of parametric modulation of reaction time were tested at group level using one-sample t-test within the areas which revealed warning related activation in the above basic analysis. The statistical threshold was set to $p < 0.01$ (uncorrected) at voxel level. Second, I divided the warning trials into two groups: one was included trials which showed the faster reaction time of following Go trial (FWarning), another was constituted trials which showed the slower reaction time (SWarning). I reconstructed the design matrix using the following four types of event condition: FWarning, SWarning, Go, and NoGo. I conducted a FWarning-SWarning comparison. The contrast images of FWarning-SWarning comparison were tested at group level using one-sample t-test within the areas which revealed warning related activation in the above basic analysis.

The statistical threshold was set to $p < 0.001$ (uncorrected) at voxel level and $p < 0.05$ (corrected for multiple comparisons) at cluster level.

3. Results

3.1 Preliminary psychological testing

The mean (± standard deviation [SD]) percentages of correct responses on Go trials were 100.0 ± 0.0% for the AN task, 99.2 ± 2.8% for the AW task, 99.6 ± 2.0% for the VN task, and 99.6 ± 2.0% for the VW task. There were no statistically significant main effects of the presence of warning (two-way repeated measure ANOVA; $F[1,23] = 2.09$; $p > 0.05$), modality (two-way repeated measure ANOVA; $F[1,23] = 0.00$; $p > 0.05$), or their interaction (two-way repeated measure ANOVA; $F[1,23] = 2.09$; $p > 0.05$) on performance accuracy. The false-alarm rates (± SD) were 7.5 ± 9.9% for the AN task, 3.8 ± 6.5% for the AW task, 4.2 ± 6.5% for the VN task, and 3.8 ± 6.5% for the VW task. Similarly, there were no statistically significant main effects of the presence of warning (two-way repeated measure ANOVA; $F[1,23] = 2.53$; $p > 0.05$), modality (two-way repeated measure ANOVA; $F[1,23] = 1.42$; $p > 0.05$), or their interaction (two-way repeated measure ANOVA; $F[1,23] = 1.56$; $p > 0.05$) on the false-alarm rate. The mean RTs (± SD) for each condition were 451.5 ± 126.1 ms for the AN task, 384.9 ± 70.9 ms for the AW task, 407.9 ± 65.0 ms for the VN task, and 380.9 ± 75.3 ms for the VW task.

Significant main effects of the presence of warning (two-way repeated measure ANOVA; $F[1,23] = 40.80$; $p < 0.05$) and modality (two-way repeated measure ANOVA; $F[1,23] = 5.73$; $p < 0.05$) were found on RT, but there was no significant interaction between the presence of warning and its modality (two-way repeated measure ANOVA; $F[1,23] = 3.78$; $p > 0.05$).

3.2. fMRI study

3.2.1. Task performance

The mean (± SD) percentages of correct responses were 98.2 ± 4.5% for the visual Go trials, 90.1 ± 5.9% for the visual NoGo trials, 98.3 ± 3.1% for the auditory Go trials, and 87.7 ± 10.0% for the auditory NoGo trials. There was a significant difference in performance accuracy between the Go and NoGo trials (repeated measures ANOVA; $F[1,25] = 29.077$; $p < 0.001$), but no effect of the cueing modality ($F[1,25] = 0.629$, $p = 0.435$) or an interaction ($F[1,25] = 0.867$, $p=0.361$). The RTs (± SD) of correct responses on Go trials were 351.1 ± 58.6 ms for the visual task and 333.2 ± 29.4 ms for

the auditory task. There was no significant difference in RT between the visual and auditory tasks (two-sample t-test; $p = 0.345$).

3.2.2. Task-related activation

Irrespective of the stimulus modality, neural activation related to the warning stimuli was identified bilaterally in the ACC, left pre-SMA, and right dorsal premotor area (PMd), as well as bilaterally in the insula and thalami extending into the midbrain (Figure 2, Table 1). Typical individual data are shown in Figure 3, and time courses for these subjects are shown in Figure 4. The Go–NoGo contrast revealed activation in the left primary sensorimotor cortex and the right anterior cerebellum (Table 2).

3.2.3. Parametric modulation of reaction time

Within the activation related to the warning stimuli (Figure 2, Table 1), parametric modulation analysis revealed the increase of activation associated with the reduction of reaction time in the left ACC (Montréal Neurological Institute coordinates x, y, z: -6, 10, 52) and thalamus (-12, -18, 2) when the warning stimuli were presented

(Figure 5). In contrast, I found no warning related activation associated with reaction time positively.

3.2.4. Comparison of FWarning amd SWarning trials

To confirm the warning effect during the fMRI experiment and the result of parametric modulation, I conducted the comparison analysis between subdivided groups of warning condition (FWarning and SWarning) within the activation related to the warning stimuli (Figure 2, Table 1). FWarning-SWarnig contrast revealed the activation in the left ACC (-6, 12, 52) and bilateral thalamus (left: -12, -20, 10; right: 10, -12, 8) (Figure 6). In contrast, SWarning-FWarning contrast revealed no activation.

4. Discussion

4.1. Midbrain and thalamus

Warning stimuli activated midbrain regions, including the reticular formation and thalamus, regardless of the stimulus modality. The midbrain reticular formation is known to be related to alertness (Kinomura et al., 1996; Fan et al., 2005; see review by Raz and Buhle, 2006). In a PET study, Kinomura et al. (1996) found evidence suggesting that the midbrain reticular formation and the thalamus (intralaminar domain) are engaged in the alerting process, and reported effects in a region (centre of gravity x = –3, y = –22, z = –9) near to those observed in the present study. I also found thalamic activation in the medial dorsal thalamic nucleus (MD), the internal medullary lamina of the thalamus (iml), and the ventral lateral posterior thalamic nucleus, internal part (VLPI) (Mai et al., 2008) (local maximum, x = 10, y = –16, z = 8; x = –10, y = –16, z = 10), which was consistent with the findings of Kinomura et al (1996). Furthermore, I found thalamic activation which was larger in the trials of faster reaction time for following Go tirial than slower reaction time. In cats, midbrain reticular formation neurons directly activate iml neurons via cortical projections, and thalamic neurons

increase their firing rate during states of heightened alertness (Glenn and Steriade, 1982). Kinomura et al. (1996) utilized a sustained attention task, corresponding to intrinsic alertness. Hence, the Kinomura et al. (1996) results together with the present findings suggest that both intrinsic and phasic alertness share neural substrates in the midbrain–thalamic system.

In another fMRI investigation of attention-related processes, Fan et al. (2005) found activation in the midbrain and the thalamus during phasic alertness produced with a visual warning cue. Although they interpreted the midbrain activity as being due to modality, the observations of Fan et al. (2005) are consistent with the modality-independent activation of the midbrain associated with phasic alertness.

4.2. ACC

ACC activity has been shown to be related to arousal (intrinsic alertness) rather than modality-specific attentional processes (Paus et al., 1997). In the present study, the ACC was activated by the warning stimulus irrespective of its modality, and revealed larger activation in the trials of faster reaction time for following Go tirial than slower

reaction time. These results might therefore be involved in phasic alertness. The ACC is known to be directly responsive to aversive stimuli and to pain (Peyron et al., 2000), and is also responsive to negatively valenced information of a more abstract nature, such as errors in performance, negative feedback, monetary loss, and even social exclusion (Falkenstein et al., 1991; Gehring et al., 1993; Miltner et al., 1997; Kiehl et al., 2000; Eisenberger et al., 2003; Holroyd et al., 2003; Ito et al., 2003; Holroyd et al., 2004a; Holroyd et al., 2004b; Yeung and Sanfey, 2004; Yeung et al., 2005). The ACC responds to task difficulty and conflicts in processing. Based on these findings, Aston-Jones and Cohen (2005) suggested that the ACC is sensitive to negatively-valenced signals that serve as indices of performance-related cost, which is an important factor in decision-making and action selection.

Recent studies suggest that the alerting processes, both phasic and tonic, are related to the LC–norepinephrine (NE) system (Aston-Jones and Cohen, 2005; Raz and Buhle, 2006). According to Aston-Jones and Cohen (2005), LC neurons show two modes of activity: phasic and tonic. In a phasic mode, a burst of LC activity driven by the outcome of a task-related decision process produces a widespread but temporary

release of NE, facilitating task-appropriate behavior. Tonic activity of LC–NE neurons strongly covaries with stages of the sleep–waking cycle, and these neurons become largely silent during rapid eye movement (REM)/paradoxical sleep. Low levels of LC activity facilitate sleep and disengagement from the environment. Tonic activity of the LC is also related to tonic alertness or vigilance.

Given that the LC sends dense projections to the ACC, the involvement of the latter in intrinsic alertness (Paus et al., 1997) and phasic alertness shown in the present study might be partly explained by the bottom-up effect of the LC–NE system. However, the monkey LC also receives prominent direct input from the ACC, which appears to exert a top-down effect on the LC. Aston-Jones and Cohen (2005) proposed that the ACC controls patterns of LC activity to optimize utility on both short and long timescales. According to their hypothesis, the warning effect or phasic alertness might be represented by the coactivation of the ACC and the midbrain–thalamic system. This activation pattern is consistent with the "anterior alerting system" proposed by Sturm and Willmes (2001). They suggest that the ACC intrinsically controls the brainstem noradrenaline (NA) activation system via the reticular nucleus of the thalamus. The

ACC, as a center for the anticipation of actions and the preparation of attentional activity, exerts top-down control over the NE activation via brainstem structures. This top-down control might be mediated by the thalamus, which specifically opens "thalamic gates" in accordance with the frontal executive systems (Sturm and Willmes, 2001). In this way, information encoded in the activity of the brainstem can be directed selectively to the cortical areas that need it for specific aspects of information processing.

4.3. Pre-SMA

The SMAs, which were previously regarded as a single motor area occupying the medial part of Brodmann's area 6, are now divided into two subregions: the anterior part (pre-SMA) and the posterior part (the SMA proper) (Picard and Strick, 1996). The SMA proper, located immediately anterior of the foot representation of the M1, has somatotopically organized movement representations, as shown in non-human primates (Luppino et al., 1991; Mitz et al., 1991; Matsuzaka et al., 1992; He et al., 1995) and humans (Fried et al., 1991; Yazawa et al., 1998). The SMA proper, which tightly

interconnects with the M1 and the spinal cord (Luppino et al., 1993), probably plays an important role in the preparation and execution of motor movements. The pre-SMA in macaque monkeys corresponds to the medial cortex of area 6, 6ab, or field F6 (Vogt and Vogt, 1919; Luppino et al., 1991; Matelli et al., 1991; Luppino et al., 1993). Although the pre-SMA lacks direct connections with area M1 and the spinal cord (see also Dum and Strick, 1991; Bates and Goldman-Rakic, 1993; Luppino et al., 1993; Lu et al., 1994; He et al., 1995), it receives major inputs from the prefrontal cortex as well as the rostral premotor and cingulate areas (Matsuzaka et al., 1992; Luppino et al., 1993). Thus, the pre-SMA is likely to play a less direct role in the execution of movements.

Physiological evidence indicates that the pre-SMA might play roles in response selection, preparation (Matsuzaka et al., 1992), and planning (Hoshi and Tanji, 2004), rather than in motor execution itself. In humans, the vertical anterior commissure (VAC) line, based on the stereotaxic coordinate system of Talairach and Tournoux (1988), serves as an anatomical landmark for the discrimination of the pre-SMA and the SMA proper (Hanakawa et al., 2001). Functional neuroimaging studies suggest that the pre-SMA might be specifically associated with the free selection of actions (Deiber et

al., 1996; Lau et al., 2004a) and the preparation of motor movements (Brass and von Cramon, 2002). Attention to intention to move has been observed to accentuate activity in the pre-SMA (Lau et al., 2004b).

Lesions of the midline frontal regions, including the SMA and CMA, are known to cause loss of voluntary movement generation, which is often called abulia, or, in extreme cases, akinetic mutism (Fisher, 1983; Hallett, 2007). Although it is not clear which of these regions are critically involved, the self-initiation of movement appears to be associated with mesial motor structures. These, and the ACC, are places of information convergence for motor control, homeostatic drive, emotion, cognition, and alertness (Paus, 2001). As the ACC, midbrain, and thalamus constitute the anterior alerting system (Sturm and Willmes, 2001), and the pre-SMA is involved in response selection and/or preparation, the co-activation pattern found in the present study can be interpreted as priming of the motor selection and/or preparation processes in the pre-SMA via inputs from the anterior alerting system, resulting in the facilitation of motor processing.

4.4. Interaction of the anterior alerting system and other attentional systems

Previous investigations into a possible neural alerting system have shown that, in addition to the anterior alerting system, tasks requiring alertness activate the frontal and parietal regions (Sturm and Willmes, 2001; Fan et al., 2005). This has been explained as co-activation of the attention-orienting networks distributed in the parietal and frontal areas (Fan et al., 2005). Although several behavioral studies using tasks designed to recruit both networks functionally demonstrated no correlation between the alerting and orienting scores, suggesting that they are functionally distinct (Fernandez-Duque and Posner, 1997; Fan et al., 2002; Rueda et al., 2004; Fan et al., 2005), interactions have been observed under some conditions (Callejas et al., 2004). In the present study, activity related to the warning stimulus was isolated from the execution-related activation produced on presentation of the Go/NoGo cue. This might have eliminated the effects of cognitive components other than phasic alertness on presentation of the warning stimulus.

4.5. Other areas of activation

I observed activity related to the warning stimulus in the PMd and insula. The PMd is also known to be related to movement preparation in non-human primates (Weinrich et al., 1984) and in humans (Watanabe et al., 2002; Kansaku et al., 2005). The insula is a center for interoception and is associated with autonomic motor control (Craig, 2003). The contribution of the insula to motor function is thought to be related to the sense of "body ownership" of a movement rather than the production of the movement itself (Hallett, 2007). The insular activation observed in the present study might be the indirect result of movement preparation.

4.6. Limitations of the present study

The present fMRI experiment did not include a control condition without warning stimuli, due to the time constraints of a slow-event-related fMRI design. This made it unclear whether the warning effect was exerted during the scan session. However, the preliminary psychological testing, which compared RTs of identical task to fMRI experiment with RTs of no-waning task outside the MRI scanner by another group, showed the clear warning effect. Furthermore, I observed the ACC and thalamic

warning related activation which were associated with reduction of reaction time of Go trials. Moreover, these areas revealed the activation during the warning trials which were associated with faster reaction time of following Go trials, compared with those which were associated with slower reaction time of following Go trials. These results suggest that the warning effect was exerted in the present fMRI experiment.

The current study also used a between-subjects design, in which different groups completed tasks with different stimulus modalities, making it difficult to compare effects between stimulus modalities. Again, this was due to time limitations. As my primary interest was in investigating whether the anterior alerting system is activated by cues of different modalities, rather than comparing the degree of activation between modalities, the present results are still valid. Future studies should employ more rapid-event fMRI protocols that allow a more integrated task design including both warning and modality effects.

5. Conclusion

I showed that warning stimuli activated neural circuits associated with a midbrain-thalamic-ACC alerting system, as well as the pre-SMA, irrespective of stimulus modality. These findings suggest that the warning effect is mediated by the priming of the mesial motor structures that include the pre-SMA and ACC for the self-initiation of movement through the midbrain–thalamic–ACC alerting system irrespective of modality.

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Tables

Table 1. Warning effect within the areas activated by both visual and auditory stimuli

cluster		voxel		MNI coordinate				
P*	size	P+	T	x	y	z	Side	Location
<0.001	825	<0.001	11.8	-6	10	44	L	ACG
		<0.001	10.4	8	14	38	R	ACG
		<0.001	10.1	8	8	58	R	pre-SMA
<0.001	323	<0.001	10.2	8	-26	-4	R	midbrain
		<0.001	9.8	10	-16	8	R	thalamus
<0.001	285	<0.001	10.1	-10	-26	-8	L	midbrain
		<0.001	9.2	-10	-16	10	L	thalamus
0.007	43	<0.001	9	36	20	4	R	insula
0.003	72	<0.001	9	52	2	48	R	PMd
0.013	24	<0.001	8.9	-34	22	6	L	insula

MNI, Montréal Neurological Institute; ACG, anterior cingulate gyrus; PMd, dosal premotor coetex; pre-SMA, pre-supplementary motor area; L, left; R, right. P* corrected at cluster level, P+ FWE corrected. The areas were masked with the activated areas by both visual and auditory stimuli with P<0.05 (FWE corrected at voxel level).

Table 2. Execution effect by Go - NoGo contrast irrespective of the cue modalities

cluster		voxel		MNI coordinate				
P*	size	P+	T	x	y	z	Side	Location
<0.001	387	<0.001	11.2	-38	-20	66	L	SM1
		<0.001	11.0	-52	-20	52	L	SM1
		<0.001	9.6	-36	-22	56	L	SM1
<0.001	76	<0.001	10.8	26	-54	-26	R	cerebellum

MNI, Montréal Neurological Institute; SM1, primary sensorimotor cortex. The areas were masked with the activated areas by Go - Nogo contrast of visual and auditory studies with P<0.05 (FWE corrected at voxel level).

Figures

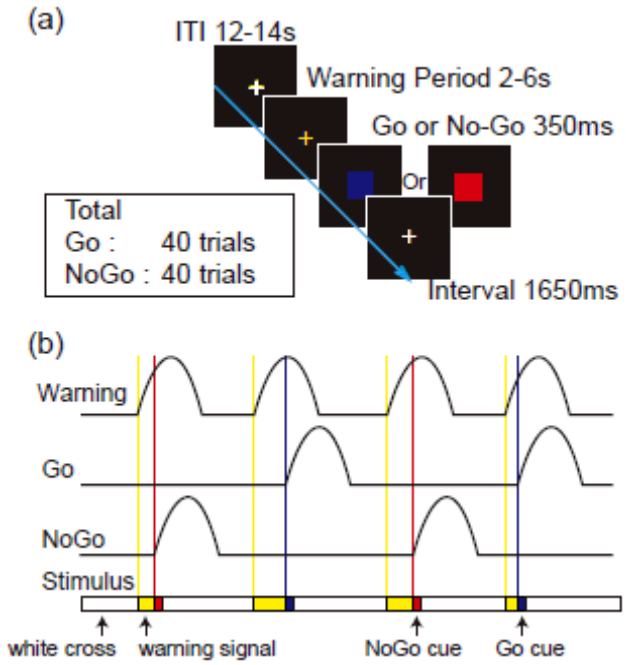

Figure 1

(a) Schematic diagram of a visual Go/NoGo task. The subjects were initially presented with the central fixation cross. After a relatively long ITI of 12–14 s, the color of the fixation cross changed from white to yellow as a warning stimulus. Following a variable time period (2–6 s), a blue or red square was presented as a Go cue or a NoGo cue, respectively. When a Go cue was presented, the subjects had to respond by pressing a button with their right thumb as quickly as possible. (b) Task design and models for analyses. The time course of the tasks is presented schematically at the bottom of the

figure. The model of the expected blood oxygen level-dependent (BOLD) signal change is presented for the event-related paradigms.

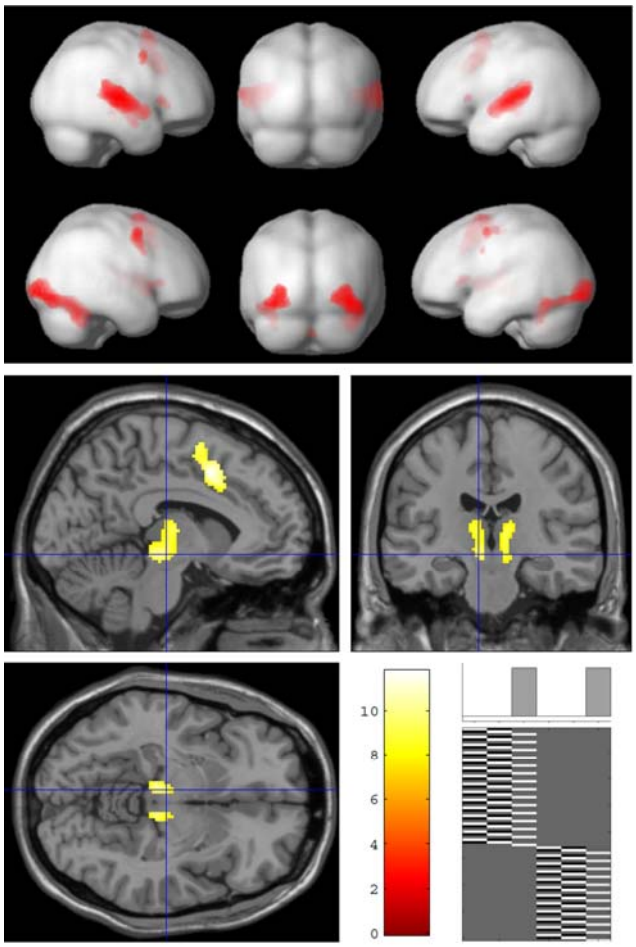

Figure 2 (Top) Auditory (upper raw) and visual warning effects superimposed on the three-dimensionally rendered average MRI. The statistical threshold was FWE corrected ($p < 0.05$ and cluster size >20 voxels). (Bottom) The warning effect irrespective of the modality of the warning stimulus superimposed on the sagittal, coronal, and transaxial images of high-resolution MRI intersected at (–8, –20, –8). The activated areas were masked with those regions activated by both auditory and visual warning effects shown

in the top panel. The statistical threshold was FWE corrected ($p < 0.05$ and cluster size >20 voxels). The color scale indicates the magnitude of the t-value. (Bottom right) Design matrix of the flexible factorial design at the second level with the contrast. From left to right, the vectors represent the visual Go, visual NoGo, visual warning, auditory Go, auditory NoGo, and auditory warning conditions.

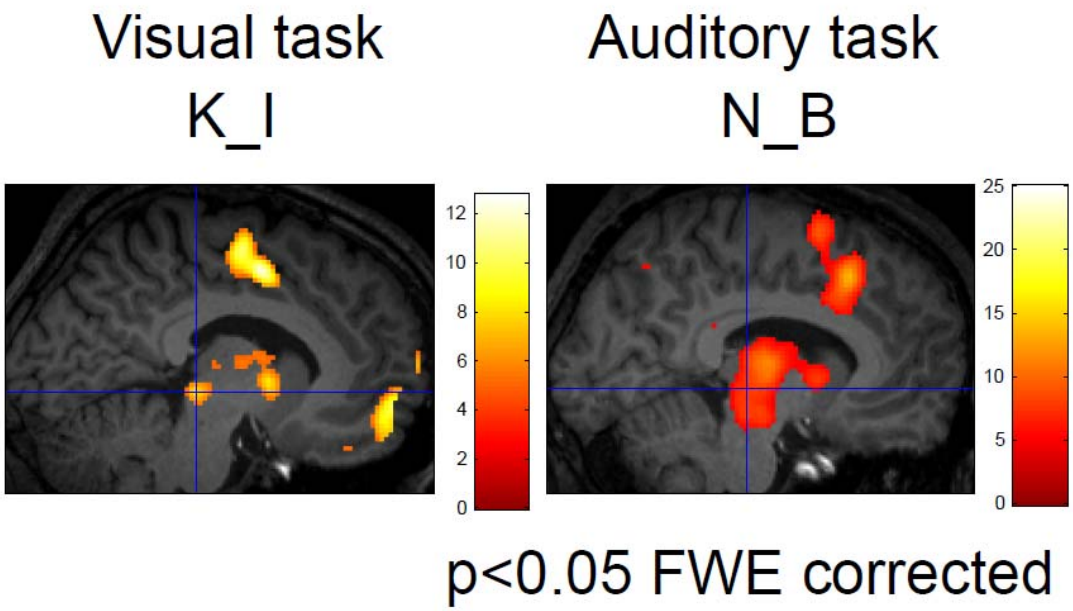

Figure 3

Individual analysis of the warning-related activation in the midbrain reticular formation, thalamus, ACC, and pre-SMA. Warning-related activation superimposed on the sagittal high-resolution MRI of each subject.

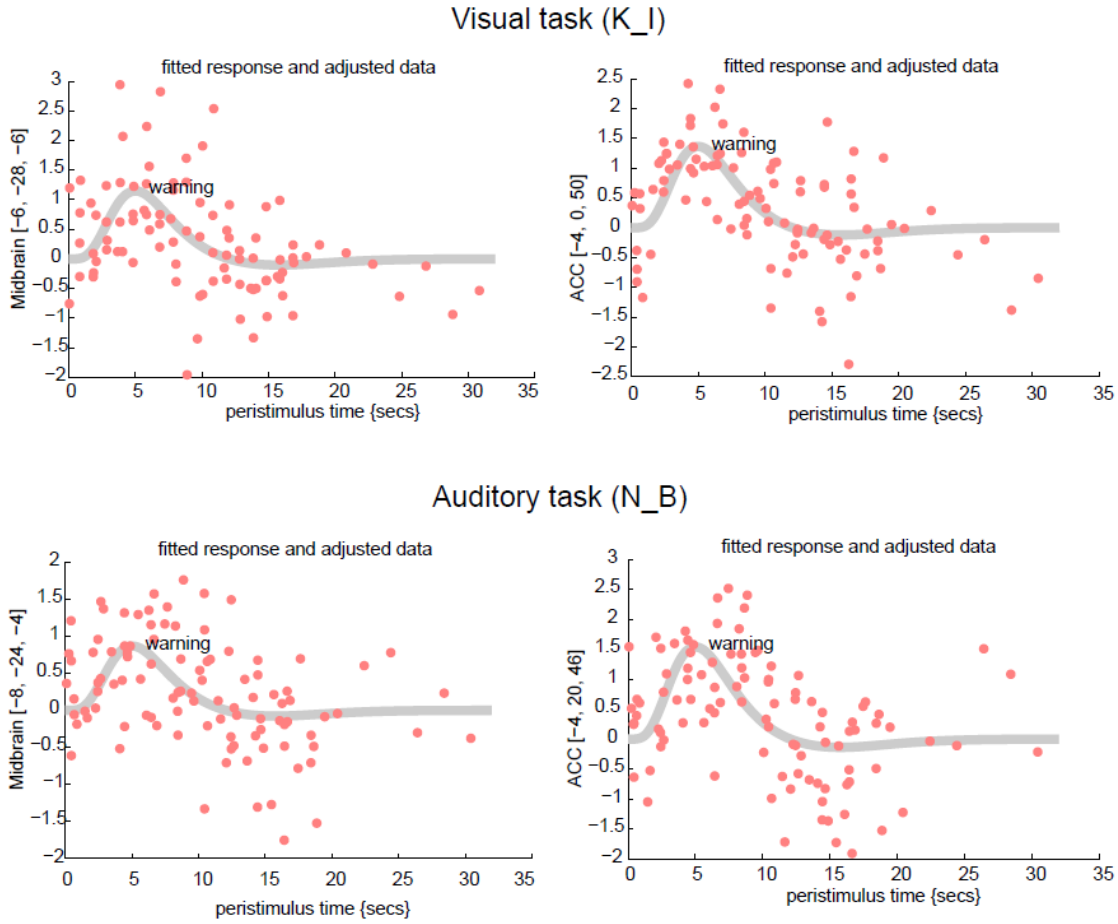

Figure 4

Individual time courses of the warning-related activation in the midbrain reticular formation were analyzed using an SPM plot. Gray lines indicate the fitted response and red dots indicate the adjusted data. The fitted response was calculated using the equation β*HRF, where β is an estimated parameter. The adjusted data were calculated using the equation β*HRF+ε, where ε is the residual.

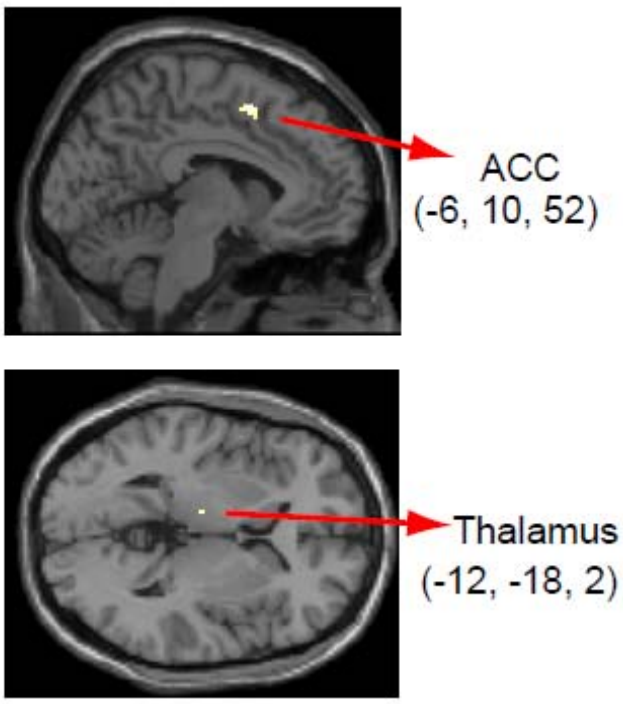

Figure 5

Warning related activations for negative correlation with reaction time of successive Go trial within warning related area (Figure 2 and Table 1) superimposed on the sagittal and transaxial images of high-resolution MRI intersected at anterior cingulate cortex (ACC) and thalamus. The statistical threshold was set at $p < 0.01$ uncorrected.

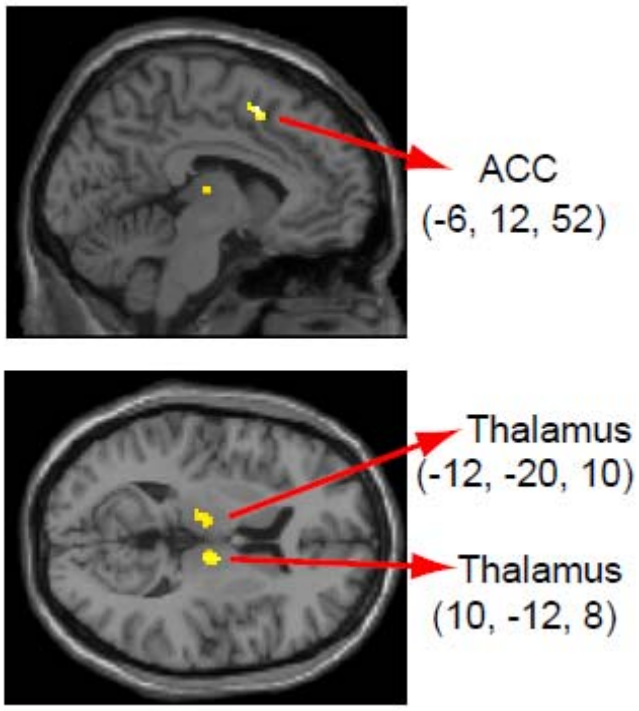

Figure 6

Activations obtained from the warning trials which revealed faster reaction time compared with those which revealed slower reaction time within warning related area (Figure 2 and Table 1) superimposed on the sagittal and transaxial images of high-resolution MRI intersected at anterior cingulate cortex (ACC) and thalamus. The statistical threshold was set at $p < 0.001$ uncorrected at voxel level and $p < 0.05$ corrected at cluster level.