

A rapid presentation event-related functional magnetic resonance imaging study of response inhibition in macaque monkeys

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Abstract

Rapid presentation event-related functional magnetic resonance imaging was applied to macaque monkeys performing a symmetrically rewarded go/no-go task, to investigate neural correlate of response inhibition. Sensorimotor activation related to the task performance was observed predominantly in the hemisphere contralateral to the response forelimb. Furthermore, no-go dominant activation possibly related to response inhibition, was observed in the ventral prefrontal cortex, in accordance with previous electrophysiological studies. These results show the feasibility of rapid presentation event-related functional magnetic resonance imaging in behaving monkeys.

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Inhibitory function is one of the characteristic prefrontal functions by which inappropriate responses to extrinsic stimuli are avoided. The go/no-go task is an effective paradigm to investigate this function [10]. Previous lesion, electrophysiological and imaging studies using this task have indicated that inhibitory function is implemented in the ventral prefrontal cortex (VPFC) of non-human primates [5, 11, 13, 14, 16, 18] and humans [7]. Although single-unit studies of monkeys can provide detailed information on neural activities at certain brain regions within the time resolution of one trial, direct comparisons between the results of electrophysiological studies of monkeys and imaging studies of humans is not straightforward because of differences in species and methodology. Functional magnetic resonance imaging (fMRI) studies of monkeys is expected to bridge the gap between electrophysiological studies of monkeys and imaging studies of humans, and has recently been applied not only to anesthetized monkeys [4,

8] but also to awake behaving monkeys [9, 17]. In this study, rapid presentation event-related fMRI (refMRI) was applied to monkeys performing a symmetrically rewarded go/no-go task to explore the neural correlates of one inhibitory function, namely response inhibition. This method provides two advantages: firstly, refMRI is a suitable experimental design for the go/no-go task paradigm, because response inhibition can be effectively induced by presenting two opposite types of trial, such as the go and no-go trials, in a random order with short inter-trial interval, and the consequent neural correlates would be transient in nature. Secondly, this method offers an experimental design similar to that used in conventional electrophysiological studies, and thus the obtained results are expected to be more comparable with those obtained from single-unit studies of monkeys.

The subjects of this study were two male macaque monkeys (*Macaca fuscata*: weighing 6.8 kg (monkey O) and 6.5 kg (monkey D)). During the fMRI sessions, visual stimuli were projected on a screen using a liquid crystal display video projector and an optic fiber-based MR-compatible lever was used for subjects' responses. The monkeys performed a symmetrically rewarded go/no-go task (Fig. 1a): when they held the lever with their right forelimbs, a warning cue in the form of a white square

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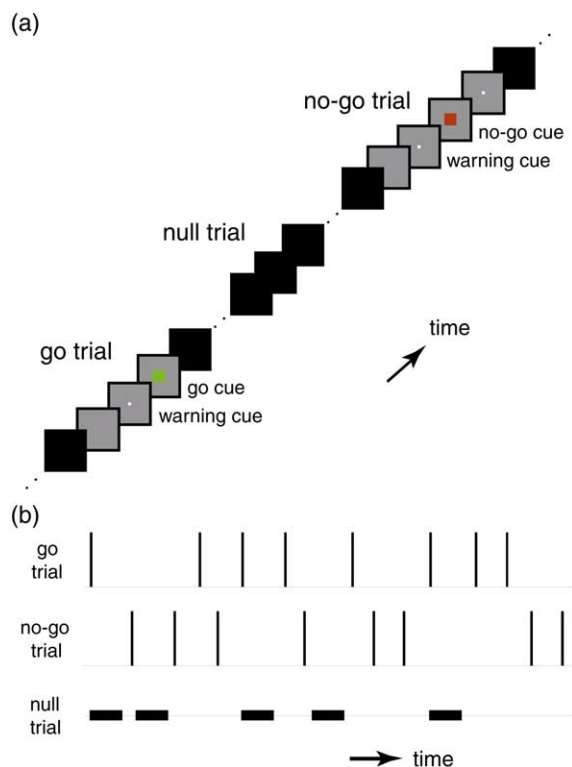


Fig. 1. Task procedures and experimental design. (a) Task sequence. When the subject held a lever, a trial started with the appearance of a warning cue (white square). Then the green and red squares indicated the go and the no-go trials respectively. The lever had to be released (go trial) or held (no-go trial) according to the color of the cue. Null trials (black screen for 3 s) were intermingled among the task presentations. (b) Schematic diagram of trial presentation. The sequence of three types of trial (go, no-go and null trials) was arranged by an optimal experimental algorithm [2,6]. Short vertical bars in the top and middle rows indicate onsets of go and no-go stimuli, respectively. Thickened parts in the bottom row indicate null trial presentations.

($0.6^\circ \times 0.6^\circ$) in the center of the screen, appeared for a variable period (0.6–3 s). A conditional cue in the form of either a green or red square ($2.8^\circ \times 2.8^\circ$), then appeared for 300 ms. If a green square (go cue) appeared, the lever had to be released within 500 ms (monkey O) or 450 ms (monkey D) after onset of the cue (go trial). If a red square (no-go cue) appeared, the lever had to be held for at least 1 s after offset of the cue (no-go trial). A correct trial was ended with a reward of a drop of liquid, and an incorrect trial was aborted without reward. To improve sensitivity to the main effects in refMRI experiments, null trials were also included where a black screen appeared for 3 s [2,6]. Each experiment consisted of identical numbers of go, no-go and null trials. Trial order was randomized and counter-balanced [2,6] and the intertrial interval was 1 s (Fig. 1b).

Imaging was performed using a whole-body horizontal 1.5 T scanner (Hitachi Medical Corp., Tokyo, Japan) equipped with a quadrature RF coil (inner diameter, 190 mm) [9]. During the imaging sessions, the monkeys' heads were immobilized with head holders. Blood oxygenation level dependent (BOLD) functional images were acquired

using T2*-sensitive two-segmented gradient echo echo-planar sequences (FOV = 128×128 mm, TR = 1.5 s, TE = 35 ms, flip angle = 70° , matrix = 64×64 , slice thickness = 2 mm, inter-slice gap = 0.5 mm, 10 transverse slices). In total, 6000 functional volumes were obtained for each monkey. In separate sessions, high-resolution 3D-anatomical images (voxel = $0.5 \times 0.5 \times 1$ mm) of the monkeys were obtained using the 3D-gradient-echo sequence.

Event-related fMRI data was analyzed using SPM99 (<http://www.fil.ion.ucl.ac.uk/spm/>, Wellcome Department of Imaging Neuroscience, London, UK) as in a previous study by the authors [9]. Briefly, the first five scans were removed for unstable magnetization of the brain tissue whilst the remaining images were realigned. Images were spatially normalized to a template constructed from 3D-anatomical images of a monkey's entire brain with interpolation of a $2 \times 2 \times 2$ mm [1]. This procedure enabled group analysis of the data from the two monkeys [9]. The functional images were smoothed using a 5-mm (full width at half its maximum) isotropic Gaussian kernel. BOLD signal changes related to both the go and no-go trials were modeled using the canonical hemodynamic response function (HRF) implemented in SPM99. The onsets of the HRF were aligned at the onset of the go and no-go cues. Error trials were independently modeled and regarded as of no interest. Individual data was estimated using a general linear model with the go and no-go trials as the main effects. Differential effects, no-go trials minus go trials and go trials minus no-go trials, were estimated using respective linear contrasts. To reveal common activations of both monkeys, individual parameter estimates were subjected to conjunction analysis [3,9]. The statistical threshold was set at $P < 0.05$, and corrected for multiple comparisons across the volume of brain under examination. The percentage of signal changes were calculated from the signal value relative to the grand mean over scans against the peristimulus time.

Both monkeys (O and D) performed the task excellently during the fMRI sessions. The mean performance levels of each monkey were over 90% ($97.2 \pm 1.9\%$ for monkey O and $94.4 \pm 2.0\%$ for monkey D (mean \pm SD)). The mean reaction times in the go trials were less than 400 ms (392 ± 43 ms for monkey O and 342 ± 34 ms for monkey D). This behavioral data confirms that the monkeys behaved in accordance with the conditional rules of the task.

Activations for go and no-go trials were almost identical, and the most prominent activation was found in the sensorimotor cortices. This activation was strongly lateralized to the left hemisphere (Fig. 2a) and extended from the anterior bank of the central sulcus to the postcentral gyrus, which corresponds to the forelimb regions identified in previous studies [4,15]. Thus, it seems that this lateralized sensorimotor activation correlates to the hold and release of the lever with the right forelimb during the trials. The percentage of signal change in the left hemisphere was

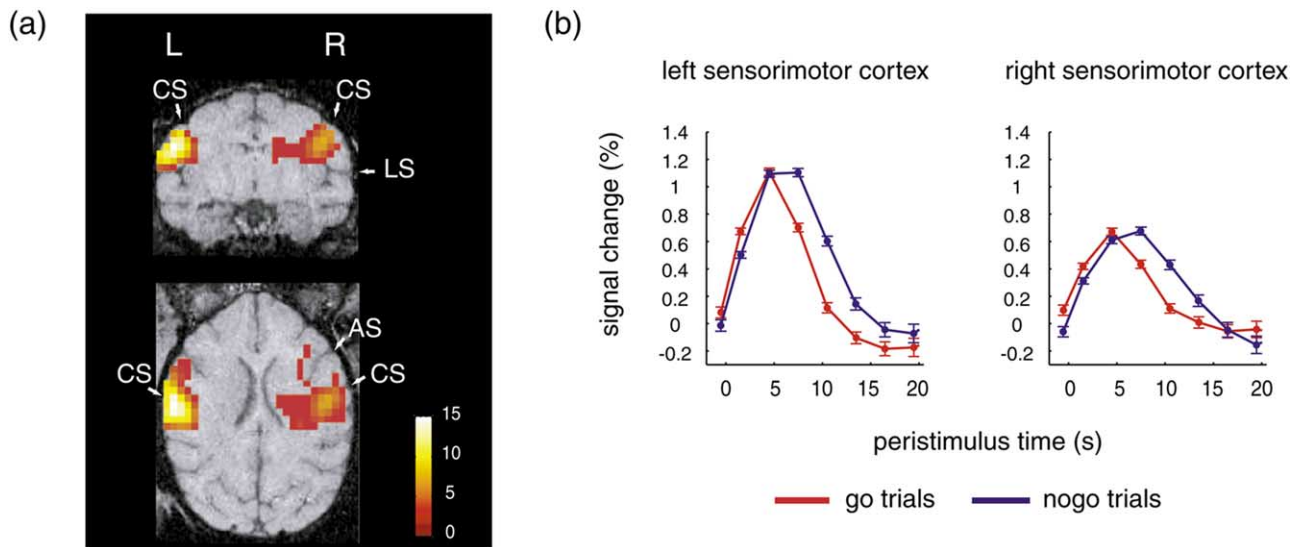


Fig. 2. Lateralized evoked responses in the sensorimotor cortex. (a) Activation of sensorimotor cortex in the go trials is presented on coronal and axial sections of normalized anatomical image ($P < 0.05$, corrected). Color bar indicates t -values. CS, central sulcus; and LS, lateral sulcus. (b) Event-related averaged time courses during the go and no-go trials at the peak voxel in left (left panel) and right (right panel) sensorimotor cortex. The fMRI signals were transiently increased time locked to the onset of the conditional cues, and peaked around 6 s later. Blue lines: no-go trials; red lines: go trials.

higher than in the right hemisphere during both the go and no-go trials (Fig. 2b). To quantify this lateralized activation, parameter estimates across all sessions were analyzed using two-way ANOVA test on laterality (left vs. right) and trial type (go trials vs. no-go trials). This analysis revealed the significance of laterality [$F(1, 79) = 42.6$, $P < 0.0001$] and the insignificance of trial type [$F(1, 79) = 0.776$, $P = 0.38$]. Other activations in the go and no-go trials were found in the bilateral ventral premotor cortex, VPFC (left during the go trials; bilateral during the no-go trials), insula (left during the go trials; bilateral during the no-go trials), right thalamus and right putamen.

To identify the brain regions associated with response inhibition, the area showing greater activation during the no-go trials compared with the go trials was analyzed. No-go dominant activation sites were found in the bilateral VPFC and the left ventral premotor cortex (Fig. 3a). To further characterize no-go dominant activation in the VPFC, the percentage of signal changes in the no-go and go trials derived from the peak voxels in the left and right VPFC were plotted (Fig. 3b). The percentage of signal increase in the no-go trials was higher than in the go trials. This difference in amplitude contrasts with sensorimotor activation where amplitudes of activation in the no-go trials and go trials were almost equivalent (Figs. 2b and 3b). To confirm the reproducibility of no-go dominant activation in each animal, single subject analysis was also performed. No-go dominant activation of the bilateral VPFC was found consistently in both monkeys O and D (Fig. 3c). No areas showing greater activation in the go trials compared with the no-go trials were found in the frontal cortex.

In this study, refMRI was applied to macaque monkeys while they performed a symmetrically rewarded go/no-go

task. Reliable transient no-go dominant activation was isolated in the VPFC using this method. In addition, sensorimotor activation related to forelimb responses was also demonstrated, and predominantly observed in the hemisphere contralateral to the response side as could be reasonably predicted. These results confirm the feasibility of refMRI in behaving monkeys.

During the symmetrically rewarded go/no-go tasks, both monkeys (O and D) inhibited their responses according to the conditional rules, which were based on stimulus-response association, not stimulus-reward association [10, 11]. Furthermore, using this refMRI design, no-go dominant activation is expected to reflect differential cognitive components of the no-go and go trials; the common cognitive components for task performance, such as visual analysis, movement selection, and motor execution were subtracted out [12]. Thus, the observed no-go dominant activation in the VPFC is most likely correlated to response inhibition time-locked to the onset of the no-go stimulus.

Previous electrophysiological studies using go/no-go tasks on monkeys reported no-go dominant single-unit activities in the VPFC [13,18]. Thus, the no-go dominant activation identified in the present study is consistent with those studies. The no-go dominant region of the prefrontal cortex identified in this study included posterior region of the inferior convexity, which was previously reported to be involved in cognitive set-shifting during the Wisconsin card sorting task (WCST) in monkeys [9]. A previous fMRI study on humans also indicated that posterior region of the right inferior frontal sulcus commonly showed activation related to response inhibition during the go/no-go tasks and activation related to cognitive set-shifting during the WCST [7,9]. These results suggest the existence of a common

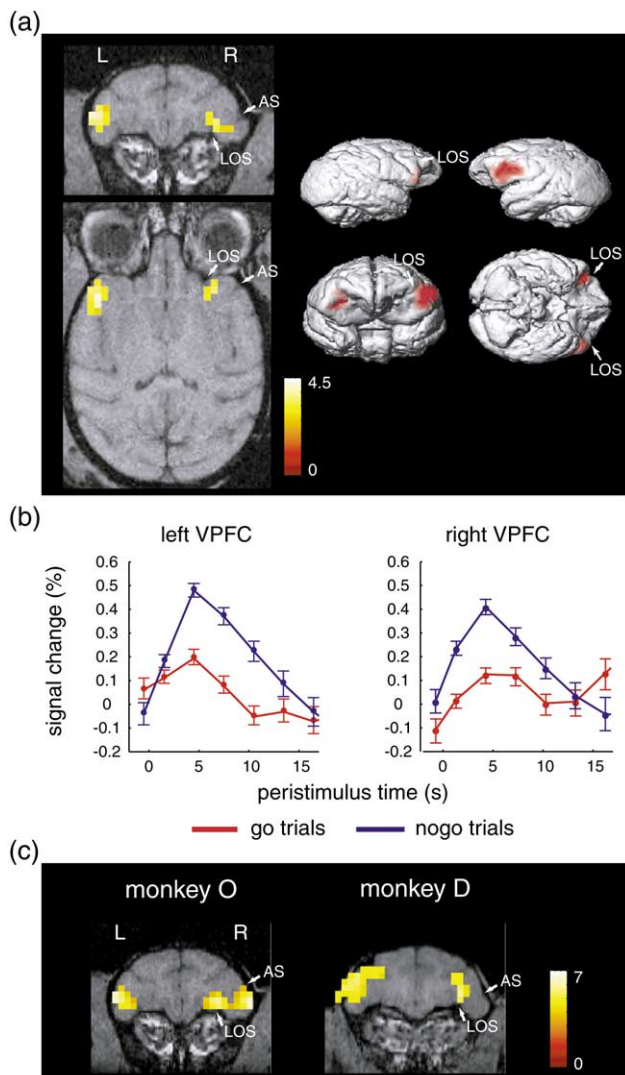


Fig. 3. No-go dominant activation in the VPFC. (a) Left panel: no-go dominant activation presented on coronal and axial sections of normalized anatomical images. Right panel: no-go dominant activation superimposed on 3D-rendered brain images ($P < 0.05$, corrected). (b) Event-related averaged time courses of percentage of signal changes at the peak voxels in the VPFC. Time courses during the go trials (red lines) and no-go trials (blue lines) are separately plotted. (c) Inter-subject reproducibility of no-go dominant activation. No-go dominant activation by single subject analysis is superimposed on coronal sections of anatomical image from each monkey ($P < 0.001$ uncorrected was used for display purpose). AS, arcuate sulcus; and LOS, lateral orbital sulcus. Color bars indicate t -values.

inhibitory mechanism in the VPFC employed during both tasks: inhibition of response to the no-go cue during the go/no-go task and inhibition of response based on previously relevant cognitive sets during the WCST. It is possible that this mechanism might be generalized across human and non-human primates.

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References

- [1] J. Ashburner, K. Friston, Multimodal image coregistration and partitioning – a unified framework, *Neuroimage* 6 (1997) 209–217.
- [2] A.M. Dale, Optimal experimental design for event-related fMRI, *Hum. Brain Mapp.* 8 (1998) 109–114.
- [3] K.J. Friston, A.J. Holmes, K.J. Worsley, How many subjects constitute a study?, *Neuroimage* 10 (1999) 1–5.
- [4] T. Hayashi, S. Konishi, I. Hasegawa, Y. Miyashita, Mapping of somatosensory cortices with functional magnetic resonance imaging in anaesthetized macaque monkeys, *Eur. J. Neurosci.* 11 (1999) 4451–4456.
- [5] S.D. Iversen, M. Mishkin, Perseverative interference in monkeys following selective lesions of the inferior prefrontal convexity, *Exp. Brain Res.* 11 (1970) 376–386.
- [6] O. Josephs, R.N.A. Henson, Event-related functional magnetic resonance imaging: modelling, inference and optimization, *Philos. Trans. R. Soc. Lond. B Biol. Sci.* 29 (1999) 1215–1228.
- [7] S. Konishi, K. Nakajima, I. Uchida, H. Kikyo, M. Kameyama, Y. Miyashita, Common inhibitory mechanism in human inferior prefrontal cortex revealed by event-related functional MRI, *Brain* 122 (1999) 981–991.
- [8] N.K. Logothetis, H. Guggenberger, S. Peled, J. Pauls, Functional imaging of the monkey brain, *Nat. Neurosci.* 2 (1999) 555–562.
- [9] K. Nakahara, T. Hayashi, S. Konishi, Y. Miyashita, Functional MRI of macaque monkeys performing a cognitive set-shifting task, *Science* 295 (2002) 1532–1536.
- [10] R. Passingham, *The Frontal Lobes and Voluntary Action*, Oxford University Press, Oxford, 1993, p. 322.
- [11] M. Petrides, The effect of periauricular lesions in the monkey on the performance of symmetrically and asymmetrically reinforced visual and auditory go, no-go tasks, *J. Neurosci.* 6 (1986) 2054–2063.
- [12] M.F.S. Rushworth, P.D. Nixon, M.J. Eacott, R.E. Passingham, Ventral prefrontal cortex is not essential for working memory, *J. Neurosci.* 17 (1997) 4829–4838.
- [13] M. Sakagami, K. Tsutsui, J. Lauwereyns, M. Koizumi, S. Kobayashi, O. Hikosaka, A code for behavioral inhibition on the basis of color, but not motion, in ventrolateral prefrontal cortex of macaque monkey, *J. Neurosci.* 21 (2001) 4801–4808.
- [14] K. Sasaki, H. Gemba, T. Tsujimoto, Suppression of visually initiated hand movement by stimulation of the prefrontal cortex in the monkey, *Brain Res.* 495 (1989) 100–107.
- [15] H. Tokuno, J. Tanji, Input organization of distal and proximal forelimb areas in the monkey primary motor cortex: a retrograde double labeling study, *J. Comp. Neurol.* 333 (1993) 199–209.
- [16] T. Tsujimoto, M. Ogawa, S. Nishikawa, H. Tsukada, T. Kakiuchi, K. Sasaki, Activation of the prefrontal, occipital and parietal cortices during go/no-go discrimination tasks in the monkey as revealed by positron emission tomography, *Neurosci. Lett.* 224 (1997) 111–114.
- [17] W. Vanduffel, D. Fize, H. Peuskens, K. Denys, S. Sunaert, J.T. Todd, G.A. Orban, Extracting 3D from motion: differences in human and monkey intraparietal cortex, *Science* 298 (2002) 413–415.
- [18] M. Watanabe, Prefrontal unit activity during delayed conditional go/no-go discrimination in the monkey. I. Relation to the stimulus, *Brain Res.* 382 (1986) 1–14.