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Reproducibility of Visual Activation During Checkerboard Stimulation in Functional Magnetic Resonance Imaging at 4 Tesla

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Purpose: To investigate the reproducibility of visual activation by checkerboard stimulation, we used functional magnetic resonance imaging (fMRI) at 4 Tesla (T).

Methods: Four subjects were studied with fMRI at 4 T during checkerboard visual stimulation. The functional images were realigned and spatially normalized to the standard brain. For each subject, statistical parametric maps were made for each study, and the reproducibility was determined based on the number of supra-threshold voxels ($Z > 3.5$, 4.5, and 5.5).

Results: The mean ratio for the number of supra-threshold ($Z > 4.5$) voxels was 0.75, and the mean ratio for the overlapping voxels was 0.61. Restricting the region of interest within the posterior half of the brain improved reproducibility values at the low threshold ($Z > 3.5$), but did not improve the values at the higher thresholds.

Conclusions: Despite the fact that more than half of the supra-threshold voxels were found to be active for the repeated scans, visual activation with checkerboard stimulation seems to be less reproducible than that by flash stimulation. **Jpn J Ophthalmol 2001;45:151–155**

Key Words: Functional magnetic resonance imaging, 4 Tesla, visual cortex.

Introduction

Reproducibility of functional magnetic resonance imaging (fMRI) has been investigated by several groups.[1–8] Previously, we studied reproducibility of visual activation in fMRI at 4 Tesla (T) using a diffuse flashing stimulus.[9] In that study, the area of activation is located on the relatively early visual cortex in the occipital lobe. Because it is known that more complex stimuli, eg, checkerboard stimuli, activate higher visual areas than simple flashing stimuli,[10] the reproducibility of visual cortex activation in the different brain regions may vary with the use of different types of visual stimuli. The aim of this study was to determine the reproducibility of visual activation in fMRI at 4 T using a checkerboard stimulus.

Materials and Methods

Subjects and Data Acquisition

Four normal volunteers (2 men and 2 women; age range, 18–24 years) gave informed consent before participating in this study. Approval of the consent and protocol for this study was given by the Committee on Studies Involving Human Beings of the University of Pennsylvania. All subjects had normal visual acuity. None of the subjects had a history of visual loss or neurologic disease.

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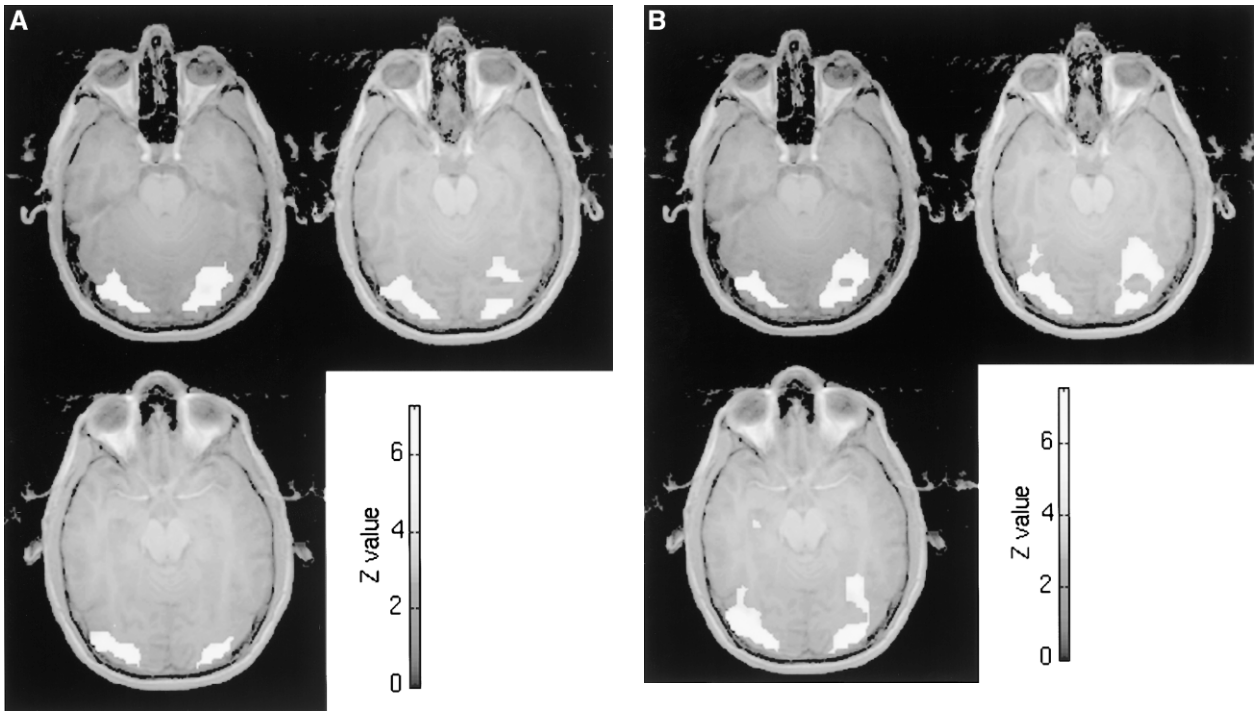

Figure 1. The SPM{Z}s overlaid on the corresponding structural images in 1 subject, showing the activated areas in the first study (**A**) and the activated areas in the second study (**B**). The left side of the brain is on the upper side of the transverse images and on the left side of the coronal images. The threshold was set at $Z > 4.5$ (corrected $P < .05$).

All studies were performed with a 4 T Signa scanner (General Electric Medical Systems, Milwaukee, WI, USA) with a quadrature head coil. 3-Dimensional T1-weighted axial images were acquired covering the whole brain for anatomic images. Subsequently, we selected a volume including the occipital lobe for functional image acquisition. Functional images were obtained using a gradient-echo echo-planar image (EPI) sequence (TR = 2000 milliseconds; TE = 28 milliseconds; matrix size = 40 × 64; field of view = 150 × 240 mm^2; 21 slices; slice thickness = 5 mm) after data for distortion correction were collected. The first 20 seconds of EPI data (10 scans) were discarded to remove the magnetic saturation effects.

The acquisition period of 120 scans of functional images consisted of 12 epochs, in which 10 scans (20 seconds) of visual stimulation alternated with 10 scans (20 seconds) of visual stimulation at the rest condition (dark screen). The acquisition was repeated twice in order to evaluate the reproducibility, without taking the subject out of the scanner. The two sessions were separated by a rest period, in which the subjects stayed within the scanner. Black and white checkerboard stimuli (mean luminance 70 cd/m^2, contrast 97%, check size 0.5°), reversing at a frequency of 8 Hz, were displayed using Macstim software (David Darby, West Melbourne, Australia) on a Macintosh computer. The visual stimulus was presented on a screen by a video projector (Sharp, XG-NV4SU), and the subjects looked at the screen through a mirror fixed to the head coil. The diameter of the visual stimuli was 4.2°. Subjects were instructed to fixate on the center of the stimuli.

Data Analysis

Data analysis was performed on UNIX workstations with IDL (Interactive Data Language Boulder, CO, USA) and SPM96 (Wellcome Department of Cognitive Neurology, London, UK) packages. First, the functional images of each subject were analyzed individually. The EPI images were realigned using a six-parameter (three translations and three rotations) rigid body transformation to the first volume. Following the motion correction, the images were transformed into the anatomical space of Talairach and Tournoux.[11] This spatial normalization routine was performed by minimizing the sum of squares difference between the anatomic images and the T1 template, using an eight-parameter affine transformation. The normalization parameters from the ana-

Table 1. Peak Activation in Visual Cortex

	1st Study Z-Score		2nd Study Z-Score	
Subject	Right	Left	Right	Left
1	6.89	7.28	6.69	7.30
2	8.07	7.71	7.69	7.51
3	6.79	6.58	7.44	6.98
4	7.18	7.25	7.52	7.20

tomic images were applied to the functional images. Data were spatially smoothed with a Gaussian filter (full width at half maximum = 8.0 × 8.0 × 10.0 mm). A box-car delayed by 6 seconds and temporal smoothing were used. *T*-statistics were calculated for each voxel and then transformed into Z-values (SPM{Z}).[12] A contrast was made for the signal intensities during visual stimulation versus those at the rest condition. The activation maps were overlaid on the T1 template of SPM96.

To assess the reproducibility of the activated areas, the ratio for the number of voxels ("R_{size}")[4,5] and the ratio for the common areas of the two studies ("$R_{overlap}$"[4,5] were calculated. The Z-maps were restored in IDL and thresholded at the Z-value of 3.5, 4.5, and 5.5. In addition, the reproducibility in the posterior half of the brain was also obtained by restricting the search region with a region of interest (ROI) drawn on the SPM template. These measurements were done in IDL, and the latter measurement was performed after the data were normalized to standard space.

Results

Activated Areas During Visual Stimulation

In all subjects, the bilateral striate and extrastriate visual cortex was activated during checkerboard stimulation (Figures 1A,B and Table 1). The number of supra-threshold voxels varied between subjects. Although activation of the frontal lobe was observed in several subjects, the activation seemed to be much less consistent in comparison with visual cortex activation across studies within a subject.

Reproducibility of Activation

The numbers of supra-threshold voxels and the reproducibility values are shown in Table 2. The ratio of number of supra-threshold ($Z > 4.5$) voxels for the repeated scans (R_{size}) in four subjects ranged from 0.60 to 0.89 (average = 0.75). The ratio of common area for the repeated scans ($R_{overlap}$) ranged from 0.56 to 0.66 (average = 0.61).

Table 2. Reproducibility of the Area of Activation

	Number of Voxels			Reproducibility Indices	
Subject	1st Study	2nd Study	Overlap	R_{size}	$R_{overlap}$
	$Z > 3.5$				
1	214	94	78	0.61	0.51
2	1915	703	649	0.54	0.50
3	450	250	175	0.71	0.50
4	722	1464	702	0.66	0.64
			Average	0.63	0.54
	$Z > 4.5$				
1	80	64	46	0.89	0.64
2	987	424	395	0.60	0.56
3	114	145	86	0.88	0.66
4	397	894	376	0.62	0.58
			Average	0.75	0.61
	$Z > 5.5$				
1	33	30	20	0.95	0.63
2	529	265	228	0.67	0.57
3	34	73	29	0.64	0.54
4	193	451	180	0.60	0.56
			Average	0.71	0.58

The numbers of supra-threshold voxels and the reproducibility values within the posterior half of the brain are shown in Table 3. When comparing the total activated areas to the area within the posterior brain, the reproducibility values were significantly higher for the posterior brain at the threshold Z-value of 3.5 (two-tailed paired *t*-test, $P < .05$). However, there was no statistically significant difference for the comparison of the reproducibility values of the whole brain activation and the posterior brain activation at the higher Z-values.

Discussion

We studied the reproducibility of visual activation in fMRI at 4 T by a checkerboard visual stimulus, which is probably the most common visual stimulus in fMRI. Most of the previous reports measured the reproducibility in visual activation of fMRI using flashing goggles, except for a recent fMRI study at 3 T which used checkerboard stimulation.[7] Therefore, the reproducibility of fMRI has been investigated mainly in the primary visual cortex. The conclusion varies among the groups, particularly reflecting the difference in the procedures, but most researchers have concluded that the reproducibility of visual activation in fMRI is acceptable. In a previous study, we measured the reproducibility of fMRI at 4 T by flash stimuli using a similar procedure.[9] In that study, "Rsize" ranged from 0.88 to 0.97 and "Rover-

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Table 3. Reproducibility of the Area of Activation Within Posterior Half of the Brain

Subject	Number of Voxels			Reproducibility Indices	
	1st Study	2nd Study	Overlap	R_{size}	$R_{overlap}$
1	205	115	90	0.72	0.56
2	1456	751	722	0.68	0.65
3	373	250	181	0.80	0.58
4	655	1210	637	0.70	0.68
			Average	0.73	0.62
	$Z > 4.5$				
1	88	74	56	0.91	0.69
2	956	489	466	0.68	0.64
3	129	155	98	0.91	0.69
4	410	836	391	0.66	0.63
			Average	0.79	0.66
	$Z > 5.5$				
1	38	36	23	0.97	0.62
2	566	306	276	0.70	0.63
3	44	84	40	0.69	0.63
4	212	477	200	0.62	0.58
			Average	0.74	0.62

lap" ranged from 0.72 to 0.86 ($Z > 4.5$, $n = 5$). Therefore, while it is generally believed that checkerboard visual stimuli are "better" at eliciting fMRI responses than diffuse flash stimuli,[13] this study suggests visual activation by checkerboard may not be as stable as the activation elicited by flash stimulation.

We used the number of supra-threshold (thresholded at three Z-values) voxels to assess the reproducibility of visual activation in fMRI. A threshold is necessary to define areas of activation in most fMRI experiments, and the number of supra-threshold voxels is often used in the assessment of cortical activation. We can roughly assume that local neural activity and regional cerebral blood flow response are parallel to each other. In fMRI, one voxel should include a large number of neurons, and the response at one particular voxel may depend on the ratio of neurons involved in the task. Therefore, neural activation at a different level can result in different numbers of supra-threshold voxels. Alternatively, fMRI may fail to show identical results in terms of the number of supra-threshold voxels even when there is actually a similar amount of neural activity between two studies.

The interaction between the threshold and reproducibility values has been reported,[5] and we used three height thresholds in this study. The best reproducibility value was found at the Z threshold value of 4.5 for both R_{size} and $R_{overlap}$. This is not in accordance with our previous report,[8] which showed the best reproducibility at $Z = 3.5$ among the three thresholds. The use of magnets at different field strengths and the different visual stimulus may account for the difference in the threshold for maximum reproducibility.

We observed some areas of activation outside the occipital cortex. Some subjects had frontal lobe activation, but the activation was not as reproducible as the visual cortex activation. This finding is obvious from visual inspection, and is confirmed by the improvement of the reproducibility values when the ROI was used to restrict the search region. However, the use of ROI did not change the reproducibility for higher Z thresholds. This suggests that the magnitude of frontal lobe (and some part of temporal lobe) activation was not as high as that in the posterior brain.

While simple flash stimuli can activate the extrastriate visual cortex as well, most activated areas lie on the primary visual cortex. In contrast, checkerboard stimulation activates both the striate and the extrastriate cortices. While a number of factors may contribute to the variability of fMRI responses (for instance, fatigue and habituation may decrease cortical activation[14]), our results seem to suggest that the reliability of extrastriate cortex activation is less than that of striate cortex activation. A direct comparison between simple and complex visual stimulation in the same subjects may provide more robust evidence for our hypothesis. In addition, it remains to be studied whether smaller or larger cortical activation can result in a different level of reproducibility in fMRI using other visual stimuli.

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