

Functional mapping of the human colour centre with echo-planar magnetic resonance imaging

KUNIYOSHI SAKAI¹†, EIJU WATANABE², YUKARI ONODERA³,
IDAI UCHIDA¹, HIDEKI KATO¹, ETSUJI YAMAMOTO³,
HIDEAKI KOIZUMI³ AND YASUSHI MIYASHITA¹

¹ Department of Physiology, School of Medicine, University of Tokyo, Tokyo 113, Japan

² Department of Neurosurgery, Tokyo Metropolitan Police Hospital, Tokyo 102, Japan

³ Central Research Laboratory, Hitachi, Ltd., Tokyo 185, Japan

SUMMARY

Clinical studies of cerebral achromatopsia have suggested a colour centre in the human fusiform gyrus. By using functional magnetic resonance imaging, we examined whether the fusiform gyrus shows activity correlated with the perception of colour. We tested three stimulus conditions in which the subject maintained fixation: (i) a circular array of six coloured circles; (ii) the same as (i) except that each circle is equiluminant grey with its colour counterpart; and (iii) the same as (i) plus a clockwise shift of circles to neighbouring positions every 1 s. After termination of the stimulus, the subject perceived an after-image of circles with complementary colours in (i), but not in (iii). In condition (i), we found a focal signal increase in the posterior part of the fusiform gyrus. In condition (ii), the activation in the same locus during the stimulation period was weaker than that in (i). In condition (iii), the signal intensity after termination of the stimulus was weaker than that in (i). The colour effect and after-effect on activation of the fusiform gyrus observed here suggest its critical role in human colour perception.

1. INTRODUCTION

In the light of current knowledge in cognitive neuroscience, cerebral achromatopsia is regarded as one piece of evidence for functional specialization in the human cerebral cortex, in that patients with bilateral damage to lingual and fusiform gyri suffer from complete loss of colour sense in the whole visual field (reviewed in Meadows 1974; Zeki 1990). Because heavy concentrations of colour-coded cells were found in visual area V4 of the macaque cortex, this colour centre in man has been proposed as human V4 (Zeki *et al.* 1991; Zeki 1993). It should be noted that monkey V4 is neither anatomically nor functionally a homogeneous field, and it consists of at least two subdivisions that may correspond to multiple roles of monkey V4 other than colour processing (Zeki 1977; DeYoe *et al.* 1994). The correspondence between the putative human V4 and monkey V4 remains a controversial issue (Zeki 1993; Merigan 1993; Ungerleider & Haxby 1994), and the crux of this debate stems from the question of whether V4 ablation in monkeys produces a condition that mimics achromatopsia (Wild *et al.* 1985; Heywood & Cowey 1987; Heywood *et al.* 1992; Walsh *et al.* 1993). Therefore it is very important to establish the functional role of the human fusiform gyrus in colour perception.

Colour effects on the activation of visual cortices have been examined in normal subjects by positron emission tomography (PET) (Lueck *et al.* 1989; Corbetta *et al.* 1990; Guylás & Roland 1991) and visual evoked potential (VEP) studies (Plendl *et al.* 1993; Buchner *et al.* 1994). However, these studies used averaging across subjects to improve signal-to-noise ratios. Recent development of the non-invasive methods of functional magnetic resonance imaging (fMRI) has enabled human brain mapping in a single subject (Kwong *et al.* 1992; Ogawa *et al.* 1992). This technique has been applied to the demonstration of retinotopy (Schneider *et al.* 1993; Sereno *et al.* 1995) and somatotopy (Sakai *et al.* 1995a) in early sensory cortices, but there has been no fMRI study on the colour perception in higher visual cortices.

One significant advantage of fMRI is the application of an ultra-fast echo-planar imaging (EPI) sequence (Stehling *et al.* 1991), which improves temporal resolution and thus enables dynamic mapping of some cortical processes. Although the timescale of milliseconds at which cortical neurons fire cannot be reached by fMRI because of the slow haemodynamic response, events lasting for seconds can be mapped effectively. Therefore, this seems a promising approach to testing psychophysical phenomena with a time constant of seconds. In the present fMRI study, we examined the cortical mechanisms related to the negative after-image, taking advantage of the fact that this visual after-effect lasts for at least 15 s after termination of colour pattern presentation. We further

† Present address: MGH-NMR Center, Massachusetts General Hospital and Harvard Medical School, Charlestown, Massachusetts 02129, U.S.A.

tested whether activation of the fusiform gyrus correlated with the perception of colour and its after-image. A part of this study has been reported in abstract form (Sakai *et al.* 1994, 1995*b*).

2. MATERIALS AND METHODS

(a) Subjects

Six normal right-handed subjects were examined in this study. The subjects were in a supine position in the magnet, and their heads were carefully immobilized with padding inside the radio-frequency (RF) coil. Extraneous sounds were dampened with plastic moulds placed in the ear canals. All subjects gave their informed consent. Approval for these human experiments was obtained from the institutional review board of the University of Tokyo, School of Medicine.

fMRI procedures were similar to those described elsewhere (Sakai *et al.* 1995*a*). We used a 1.5 T fMRI system equipped with EPI sequences, which was developed at Hitachi Central Research Laboratory. The gradient coils of elliptic cylinder shape were designed to reduce the power requirements for gradient field drivers. Their maximum strengths were $G_x = 24 \text{ mT m}^{-1}$, $G_y = 36 \text{ mT m}^{-1}$, $G_z = 40 \text{ mT m}^{-1}$. The RF coil (quadrature coil) of cylinder shape (diameter 26 cm; length 20 cm) was optimized to enhance its sensitivity without degrading the local homogeneity of the RF field in cortical regions. An RF shield (diameter 33 cm; length 25 cm) surrounded this RF coil.

(b) Visual stimuli and paradigm design

Computer graphics-based visual images were presented on a rear-projecting screen (diagonal size 125 cm; CINE25, Dlapar Screen Corporation, Inc., Indiana, U.S.A.) near the subject's feet with a liquid crystal projector (XV-E300, Sharp Corporation, Osaka, Japan). The subject viewed the stimuli through prism glasses (visual angle 12°). Visual stimulus presentation was controlled by a graphic display controller (FBX24, TEXNAI, Inc., Tokyo, Japan), and it was synchronized with echo-planar (EP) image acquisition. The main stimuli used in this study are shown in figure 1.

A single trial consisted of the first resting period (30 s), visual stimulation period (30 s), and then the second resting period (at least 40 s, up to 60 s). The stimulus presented during the first and the second resting periods was a grey square (luminance 7.3 Cd m^{-2}) with a small white cross at the centre for fixation (see figure 1*b*). This amount of luminance corresponds to that of total incident light upon the screen, including very dim ambient light. The subject was instructed to maintain fixation on the central cross throughout every trial, avoiding eye movements as much as possible. We tested one of the following three stimulus conditions during the visual stimulation period. Condition (i) was a central fixation point and a circular array of six coloured circles with boundaries (see figure 1*a*). The six colours were as follows, where the first two numbers are the x and y CIE (Commission International d'Eclairage) coordinates and the third number is the luminance in Cd m^{-2} : yellow (0.443, 0.538, 20.0), magenta (0.363, 0.153, 5.5), blue (0.140, 0.062, 0.6), cyan (0.200, 0.362, 16.5), green (0.260, 0.693, 11.8), and red (0.675, 0.295, 3.5). The background was the same grey (0.280, 0.310, 7.3) as that used in the first and the second resting periods. The purpose of using this colour stimulus was to test its negative after-image, which remains even when the stimulus disappears. Because the static stimulus presentation for 30 s may cause adaptation or habituation of the cortical response, the resultant activation of visual areas would not be at an optimum. To prevent this,

boundaries were flickered between black and white every 60 ms, while the colour circles themselves remained static. Flickering boundaries can create a more vivid and salient impression, and they produce illusory wobbling movement of every circle, without affecting the negative after-image. The second resting period following this stimulus was the same physical presentation of a grey square with a cross, as during the first resting period. Nevertheless, the subject can perceive an after-image of circles with complementary colours that are only phenomenally present. With stable fixation, six colours can remain for about 15 s, and some of these colours can last as long as 50 s (see tables 1 and 2). This negative after-image can be demonstrated using figures 1*a*, *b* with reflected lights (see instruction in the legend).

Condition (ii) is the same as the first one except that each circle is equiluminant grey with its colour counterpart (see figure 1*c*). The equiluminant grey values were determined for each subject in a calibration experiment, using a standard flicker photometric test. During flicker photometry, a colour square and a grey square are alternately presented at a rate of 28.2 Hz, and the perceived degree of flicker becomes minimal when the variable grey level meets the equiluminance point. This procedure was repeated for each of the six colours in turn, and a grey replica of the colour pattern for each subject was constructed. Because the arrangement of grey circles was exactly the same as the colour ones, and the borders flickered between black and white in the same manner, the only difference between the colour and the grey patterns was the presence of colour in the former. The colour effect is represented as condition (i)–condition (ii). During the second resting period of this grey vision, the subject can perceive the negative after-image, but its duration is very short and it has no colour at all. If the circle was white, its after-image becomes black, and vice versa.

Condition (iii) is the same as the first one plus a clockwise shift of circles to neighbouring positions every 1 s. The idea behind this, is to let each retinal position be exposed by all six colours during the stimulation period for 30 s. Therefore, one part of the visual field is equally exposed by six colours, five times. During the second resting period, the subject perceived no after-image at all. The colour after-effect is represented as condition (i)–condition (iii) during the second resting period. During the visual stimulation period in condition (iii), an additional local luminance change is introduced which is absent from condition (i). However, there was no significant difference in the observed signals during the stimulation period (see table 2), which confirmed that these stimuli were equally effective.

To ensure that attention was paid to the visual stimulus and to the after-image, each subject was required to push a handheld switch when it was perceived that the after-image completely disappeared. The duration of the after-image, between termination of pattern presentation and the subject's response, was measured at each trial. Although we did not monitor the subject's eye movements, the after-image duration was a very sensitive indicator of fixation performance. According to separate measurements of the after-image duration, in which fixation stability was voluntarily modified, we found that the better the fixation, the longer the after-image duration.

(c) Data collection procedure

Each session consisted of a series of 14–24 trials, and two of the three stimulus conditions (see §2*b*) were tested in alternate trials within a single session for each subject. For the functional imaging, we used a blipped, gradient-echo EPI pulse sequence with a flip angle (FA) of 90° , to give T_2^* weighting that is sensitive to local deoxyhaemoglobin levels.



Figure 1. Visual stimuli for testing colour effects and colour after-effects. (a) colour stimulus. A small white cross at the centre is a fixation point. The boundaries of six circles were flickered between black and white (i.e. changed from black to white and back again) every 60 ms, whereas the colour circles themselves remained static. (b) resting stimulus. A single trial consisted of the first resting period with the stimulus (b) (30 s), visual stimulation period with the stimulus (a) (30 s), and the second resting period with the stimulus (b) (at least 40 s, up to 60 s). The stimuli (a) and (b) appeared on the centre of the screen, with the fixation cross in the same place. (c) grey stimulus. Each circle is equiluminant grey with its colour counterpart, determined by standard flicker-photometry criteria for each subject. This grey stimulus was replaced with the colour stimulus of (a) in stimulus condition (ii) (see §2). The reader can test negative after-images with these stimuli. First, keep your eyes on the central cross in (a) for at least 30 s. Do not blink if possible. The effect depends

The central $(k_x, k_y) = (0, 0)$ echo was formed 10 ms (echo time (τ_E)) after the initial $\pi/2$ pulse (Turner *et al.* 1991), and the total data acquisition time for each slice image was 60 ms with the half Fourier method. Horizontal EP slices of 1 cm thickness (field of view (FOV), $260 \times 260 \text{ mm}^2$; in-plane resolution, $2 \times 4 \text{ mm}^2$) were obtained every 2 s (repetition time (TR)) continuously during each trial. The in-plane size of each pixel in an EP image was $2 \times 2 \text{ mm}^2$ after linear interpolation. A spin-echo (SE) image with T_1 weighting (TR = 500 ms; $\tau_E = 30$ ms) of the corresponding horizontal slice (FOV, $260 \times 260 \text{ mm}^2$; in-plane resolution, $1 \times 1 \text{ mm}^2$) was taken every 12 trials. This SE image was used for the confirmation of the head position and for the anatomical registration of activated regions. Each pixel in an EP image corresponds approximately to the pixel in the same position of an SE image, because the same field of view and slice parameters were used for the same gradient coils. The expected geometric distortion for EP images was estimated in our previous report (Sakai *et al.* 1995a).

Prior to EP scans in a single session, we first obtained the SE image of a mid-sagittal section for the same subject, and selected a horizontal plane to be observed. Next we obtained the SE image of the horizontal plane and identified the fusiform gyrus on this SE image. The posterior part of the fusiform gyrus is located in the inter-hemispheric side of the occipital region, and adjacent to the posterior edge of the cerebellum. Talairach coordinates x and y (Talairach & Tournoux 1988) were measured directly on the horizontal SE image for each subject, without employing standardization of individual images. The centre of the thickness of the horizontal slice corresponds approximately to $z = -10$ mm in Talairach coordinates. Then we acquired the final EPI data using this single slice position. To avoid the contamination by ghost images, the extent of EP images was restricted to regions posterior to the cerebral peduncle, by presaturating the anterior part of the brain.

(d) Data analysis

We identified areas with signal increase related to the visual stimulation by the following procedures. Images in the first resting period of each trial ($i = 1, 2, \dots, n$) were averaged as a baseline x_i^0 for each voxel. The first five images at the sampling onset were excluded from averaging to ensure the stable resting condition of brain tissues as well as the subject's alertness. Thus the first resting period, the stimulation period, and the second resting period correspond to image numbers 1–10, 11–25, and 26–45 (or 26–55), respectively. For each voxel at image number N , a signal change from the averaged baseline $\Delta x_i^N = x_i^N - x_i^0$ was calculated. Then the mean and variance of a normalized signal change $\Delta x_i^N / x_i^0$ were obtained across trials within a single session. Statistical significance of signal increase (null hypothesis: the mean of $\Delta x_i^N / x_i^0$ is zero) was assessed with one-tailed Student's t -test for each voxel. Voxels with insignificant ($p > 0.05$) and negative t -values were zero-filled, because negative correlations were not investigated in this study. These t -values were then superimposed on the corresponding anatomical SE image with a colour scale. This colour map is called here the dynamic t -map for image number N (see figure 2a). For each

on how well you can maintain your fixation. Next, fixate at the central cross in (b). You will see the complementary colours, which are completely different from the colours of the previous pattern. If the after-image disappears suddenly, it is because of your eye movements. Try to fixate again at the centre. Using (c) and (b), grey after-images can be tested in the same manner.

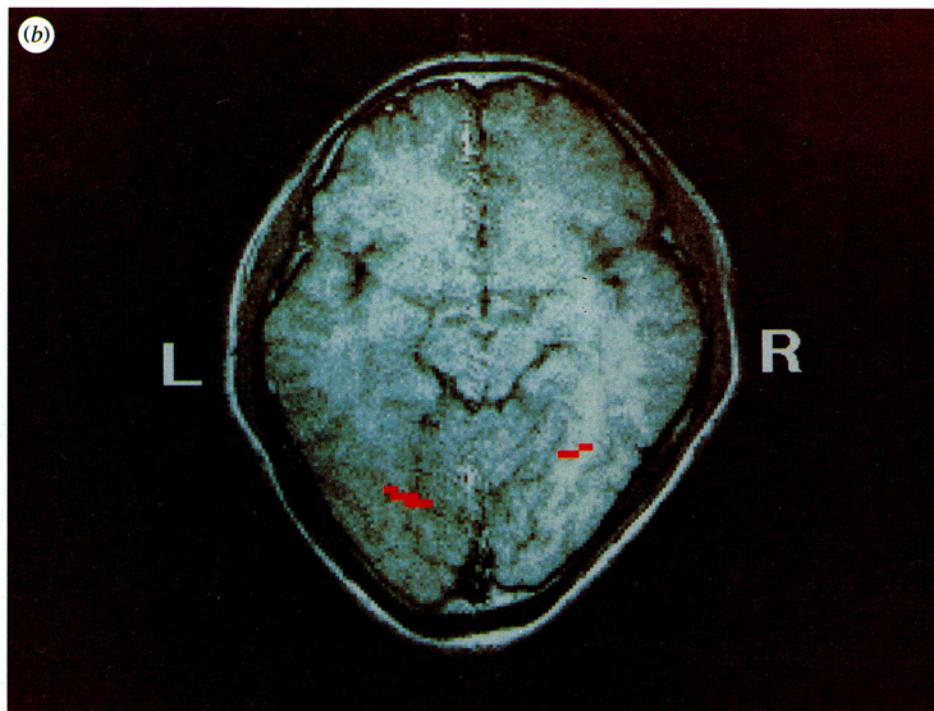
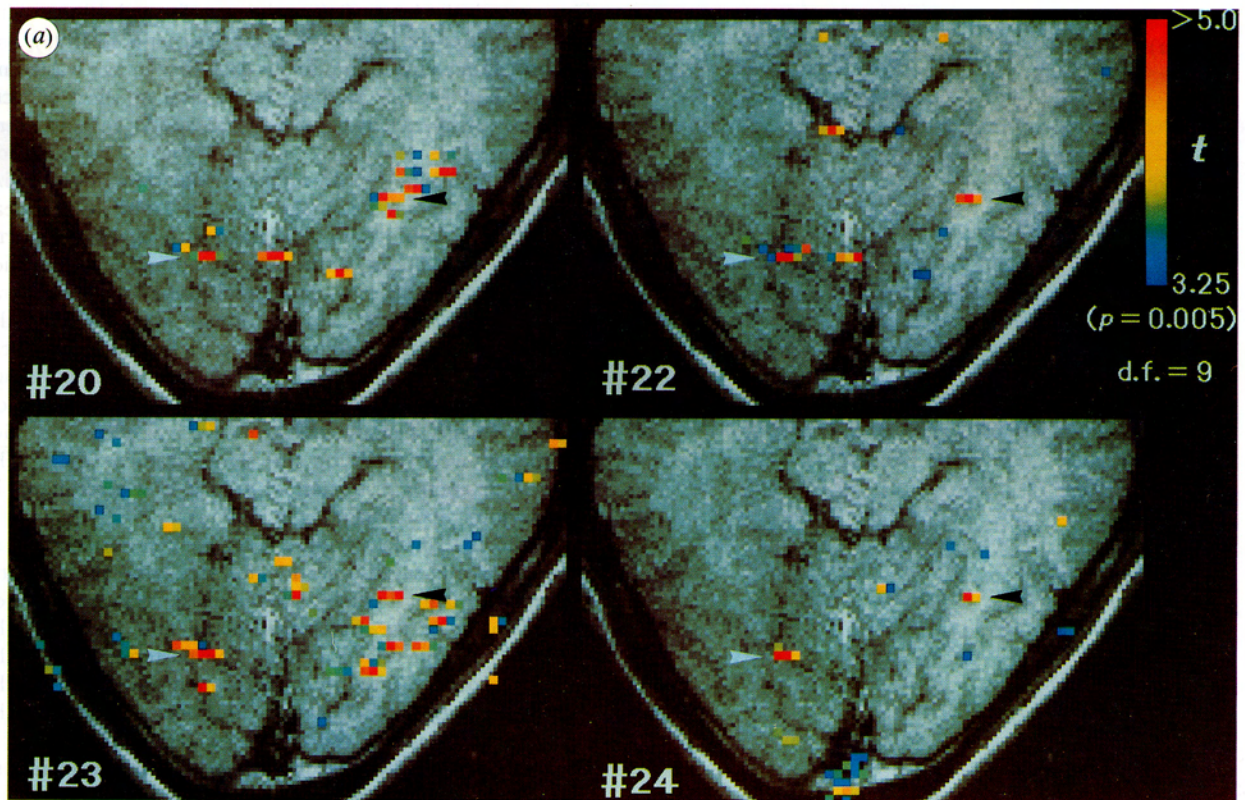


Figure 2. Statistical methods to detect significant activation. (a) Dynamic t -maps. In the occipital half of a horizontal SE image shown in figure 2b, medial structures are cerebral peduncle and cerebellum. Four dynamic t -maps during the stimulation period were shown. In each map, t -values are colour-coded using the colour scale shown in the figure. Coloured areas are all significant ($p < 0.005$), but some of them might be due to transient noises. Consistently activated areas can be evaluated by examining a sequence of dynamic t -maps. A red spot in the left hemisphere (white arrowhead) is active in all four t -maps. There is another consistent spot (black arrowhead) in the right hemisphere. These activated areas are very focal. (b) Region of interest (ROI) for the posterior part of the fusiform gyrus (PFG). L, left, R, right. From data shown in figure 2a, the region of interest was determined. After setting the same threshold of t -values ($p < 0.005$), all contiguous significant voxels were combined to make a single ROI. Left and right ROIs contain ten voxels and five voxels, respectively. We assume that these two areas correspond to the bilateral PFG, because

session, a set of dynamic t -maps reveals temporal changes of activation that may correlate with stimuli. This analysis method does not require correction for the inherent temporal correlations within each trial.

Consistent activation was evaluated by examining a sequence of dynamic t -maps. When the same voxels in the fusiform gyrus were found to be significant ($p < 0.05$) for at least three out of 15 dynamic t -maps during the stimulation period, all contiguous highly significant voxels ($p < 0.005$ or 0.001) were combined to make a single region of interest (ROI) in each hemisphere. If the number of voxels within a ROI was less than three, that ROI was regarded as noise and discarded. The number of voxels in a single ROI ranged from three to 16. If there were two or more ROIs in the fusiform gyrus, one ROI with the highest t -values was used. Figure 2*a* exemplifies this procedure to obtain the resultant ROIs shown in figure 2*b*. The positions of ROI centres were reproducible for each subject (compare Talairach coordinates in tables 1 and 2 for corresponding subjects). To enhance signal-to-noise ratios, normalized signal changes for each image number were averaged across trials of the same stimulus condition in a single session (ranging from seven to 12 trials), and they were further averaged for voxels of ROI in each hemisphere. Each ROI was defined in condition (i), and condition (ii) or (iii) was compared with (i) using the same ROI. Data processing was performed on HP375 and HP720 computers (Hewlett-Packard Company, Cupertino, California, U.S.A.) with custom software.

3. RESULTS

In condition (i) (see §2), we found that the colour pattern shown in figure 1*a* produced a significant increase of signal intensity in the posterior part of the fusiform gyrus (PFG) (see figure 2*a*). Figure 3*a* shows the timecourse of activation in both sides of PFG. Shortly after the onset of stimulus, signal intensity increased and reached a plateau of about 2% level with a rise time of 4 s. The recovery time required to reach the resting level appears to be longer than the rise time. Both left and right PFG regions show similar timecourses in this case.

To examine whether the colour of the stimulus is the critical factor for this signal increase, we prepared the grey pattern which has the same luminance as the colour pattern (see figure 1*c*) for condition (ii) (see §2). We tested the colour stimulus and the grey stimulus in alternate trials in a single session. The result is shown in figure 3*b*, averaged in the right PFG region defined for condition (i) in one subject. There was no difference during the first resting period. However, there was a clear difference in signal intensity during the stimulation period and the subsequent second resting period. The colour pattern had a stronger effect on PFG activation than the grey one. Because the luminance and other parameters were exactly the same in the two cases, the only remaining factor to explain this difference is the colour effect.

This evidence of colour effect was further confirmed in other subjects (see table 1). The timecourse shown in

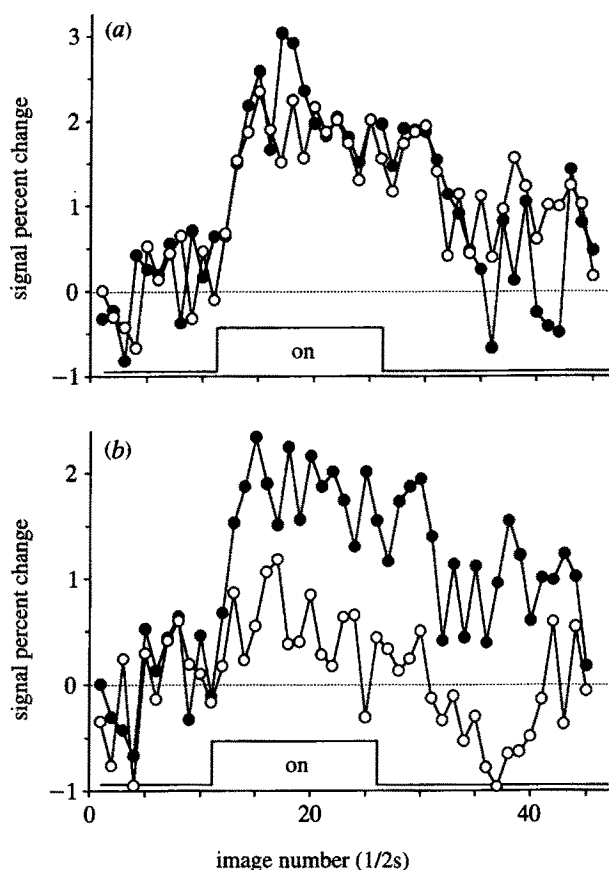


Figure 3. Colour effect on activation of the posterior part of the fusiform gyrus (PFG). (a) bilateral PFG activation. The abscissa is image number, one per 2 s. The ON period corresponds to 30 s of visual stimulation (image numbers 11–26). The ordinate is the signal percent change relative to the baseline. The filled circles denote left PFG activation, and the open circles denote right PFG activation. Clear activation in bilateral PFG was induced by the colour stimulus shown in figure 1*a*. These data were obtained after averaging ten trials for voxels of left or right ROI shown in figure 2*b*. (b) colour versus grey. The filled circles denote trials for the colour pattern (figure 1*a*), and the open circles denote trials for the grey pattern (figure 1*c*). These data were obtained after averaging ten (colour) or nine (grey) trials for voxels of right ROI shown in figure 2*b*. Note that colour stimulus elicited much stronger activation than the grey one during the stimulation period and the second resting period.

figure 3*b* is representative of all examined subjects, in that PFG response for the colour stimulus was always greater than that for the grey stimulus during the stimulation period. This difference between colour and grey was statistically significant across hemispheres, assuming that left and right PFGs are independent samples ($p < 0.001$, $t(7) = 7.19$; two-tailed t -test for self-paired data). In addition, the duration of perceived colour after-images was much longer than that of grey ones for all subjects (see table 1). The activated areas were also consistent among subjects. The variation in the Talairach coordinate x depends on the slice position

they are both in the fusiform gyrus. Although the right ROI seems to be more anterior than the left one, the smaller size of the overall right hemisphere and the similar structures of sulci and gyri indicate that these two areas are anatomically equivalent.

Table 1. *PFG activation for colour versus grey stimulus*

(L, left; R, right. Talairach coordinates of ROI centres are stated as millimetres from the anterior end of the AC-PC line and correspond to the stereotactic conventions of the atlas of Talairach and Tournoux (Talairach & Tournoux 1988). PFG response is the time integral of the per cent change in MRI signal during the stimulation period (image number 11-26), after averaging for ROI and trials of condition (i) for colour or condition (ii) for grey. After-image duration is stated as the number of seconds from the onset of the second resting period to the time when all after-images completely disappeared. Grand mean was calculated over all tested hemispheres, using mean data for subject 1.)

subject	side (L/R)	Talairach coordinates		PFG response		after-image duration	
		<i>x</i>	<i>y</i>	colour	grey	colour	grey
1	L	-23	-63	29.4	13.5	39	20
	R	+25	-50	25.5	7.3	—	—
1	L	-22	-65	26.3	19.9	51	20
	R	+22	-53	17.2	6.2	—	—
1	L	-29	-59	20.7	3.3	54	18
	R	+24	-51	12.8	1.4	—	—
Mean for 1	L	-25	-62	25.5	12.2	48	19
	R	+24	-51	18.5	5.0	—	—
2	L	-21	-68	16.7	7.1	44	24
	R	+25	-69	22.6	15.4	—	—
3	R	+21	-62	42.6	28.0	36	12
4	L	-20	-60	12.5	4.0	33	20
	R	+13	-62	14.5	8.0	—	—
5	L	-7	-67	13.4	9.4	45	10
grand mean		-18 (L) +21 (R)	-63	20.8	11.1	41	17

because the slice plane is oblique to the tentorial surface. The variation in the Talairach coordinate *y* depends on the size difference of hemispheres in addition to the slice position.

It should be noted that the time course of PFG activation for the colour stimulus shows delayed recovery to the resting level (see figure 3), which may imply the effect of after-image. To test this possibility, we devised another stimulus that actively suppresses the after-image for condition (iii) (see §2). We tested the original after-image condition and the non-after-image condition in alternate trials of a single session. The result is shown in figure 4*a*, averaged in the right PFG region defined for condition (i). There was no difference during the first resting period and the stimulation period. However, there was a clear difference in signal intensity after the stimulation period and thus during the second resting period. In the case of active suppression of after-image, the signal intensity returned to the resting level without delay. In contrast, the effect of after-image was observed as the delayed recovery of signal intensity. The difference between these two timecourses remained for at least 30 s. This difference was further clarified by subtracting the timecourse of the non-after-image condition from that of the after-image condition. Figure 4*b* shows the net effect of the after-image, which does not depend on an external physical stimulus, but depends on the internal after-effect. The positive difference clearly began to appear shortly after termination of the real pattern.

This evidence of colour after-effect was further confirmed in other subjects (see table 2). The time-course shown in figure 4*a* is representative of all examined subjects, in that PFG response for after-image

was always greater than that for active suppression of after-image during the second resting period (at least for 30 s at the beginning). This positive difference was statistically significant across hemispheres during the second resting period ($p < 0.001$, $t(7) = 7.63$; two-tailed *t*-test for self-paired data). In contrast, there was no significant difference for PFG activation during both the first resting period ($p > 0.1$, $t(7) = 1.37$) and the stimulation period ($p > 0.1$, $t(7) = -0.61$). The activated areas were also consistent among subjects. PFG activation was bilateral in some experiments, but unilateral in others (see tables 1 and 2). The detection threshold ($p = 0.05$) may mask a weaker side of activation, providing that there is asymmetry in response magnitudes. Another possibility is the difference between heights (in *z* direction) of PFG in each hemisphere, because only one slice was obtained in this study.

4. DISCUSSION

In our previous study of the somatosensory cortex with fMRI, we noted that the recovery time required to reach the resting level was more variable than the rise time, and suggested that the recovery time may correlate with the duration of after-sensation (Sakai *et al.* 1995*a*). We found that fMRI signals in PFG showed activation extending into the second resulting period following the stationary colour stimulus, but not following the rotary colour stimulus that leaves no negative after-image. To our knowledge, cortical activation that is related to the colour after-effect has not been detected in human or animal studies. Although it has been noted that after-images originate in the retina (MacLeod & Hayhoe 1974), the

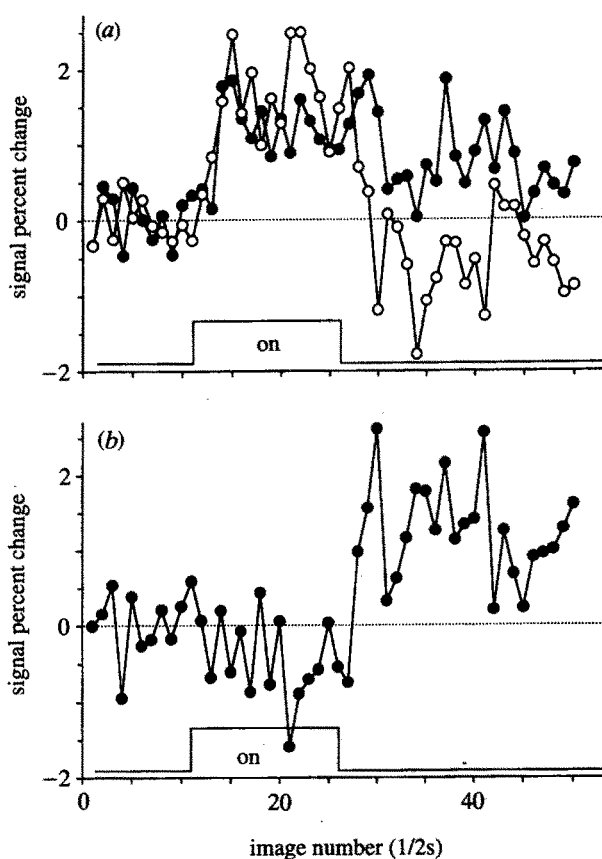


Figure 4. Colour after-effect on activation of the posterior part of the fusiform gyrus (PFG). (a) effect of colour after-image. The abscissa is image number, one per 2 s. The on period corresponds to 30 s of visual stimulation (image numbers 11–26). The ordinate is the signal percent change relative to the baseline. The filled circles denote trials with after-image and the open circles denote trials without after-image. These data were obtained after averaging nine trials for nine voxels of ROI on right PFG. Note the difference in signal changes between two conditions, which was apparent only during the second resting period. (b) net effect of colour after-image. This difference was obtained by subtracting the non-afterimage component from the timecourse of after-image condition shown in figure 4a. Note that the net effect of colour after-image started to appear shortly after the termination of the real stimulus.

perception of after-images may require central as well as peripheral mechanisms. Zeki (1983) reported that the perceived natural colour of an area and also the colour of its after-image bear no obvious relation to the wavelength composition of the light reflected from it alone (Land 1986). Therefore the opponent colour of the after-image 'must be due to central mechanisms, ones occurring after the nervous system had generated colours from wavelengths' (Zeki 1983). Schiller & Dolan (1994) showed that both peripheral and central mechanisms contribute to visual after-effects, by demonstrating partial interocular transfer of after-effects in both monkeys and humans. Our results suggest that the human PFG is a candidate area for central mechanisms in colour after-effects. It remains to be studied whether activation owing to the colour after-effect is observed in visual areas V1 and V2, because equipment limitations prevent us from acquiring large volumes of brain data. Recent fMRI

studies by Tootell *et al.* (1995) reported a motion after-effect specific to MT/V5 activation, in which sustained viewing of visual stimuli moving in a single direction results in the perception of motion in the opposite direction. Our results of a colour after-effect signal in PFG matches their result of a motion after-effect signal in MT/V5, demonstrating that the fMRI signal correlates with the perception of visual attributes.

The colour effect and the colour after-effect on PFG activation reported here, provide new supporting evidence that human PFG is a colour centre outside the primary visual cortex. Our fMRI study of normal, single subjects is consistent with the following reports. The first one is from lesion studies of cerebral achromatopsia and hemiachromatopsia that result from focal damage in lingual and fusiform gyri (Verrey 1888; Damasio *et al.* 1980; Kölmel 1988; Rizzo *et al.* 1992; Kennard *et al.* 1995). In patients with hemiachromatopsia, objects in the visual hemifield ipsilateral to a unilateral lesion appear to be of normal colour, while in the contralateral hemifield they are perceived only in shades of grey (though with little distortion of form). The second report is from PET studies, which showed activation around the collateral sulcus between lingual and fusiform gyri, in agreement with the location of lesions associated with achromatopsia. The experimental conditions in these studies were: a colour Mondrian pattern (15 multicoloured squares and rectangles) against an equiluminant grey version of the colour Mondrian (Lueck *et al.* 1989; Zeki *et al.* 1991); and selective attention to colour of a stimulus with several visual attributes against passive or divided attention (Corbetta *et al.* 1990, 1991). The third report is from VEP studies with electrodes on the scalp, which compared colour versus grey Mondrian stimuli (Plendl *et al.* 1993; Buchner *et al.* 1994). Although there was no difference in magnitude between colour and grey stimulated VEPs, source analysis on colour minus grey data isolated activity in the medial occipital region (Buchner *et al.* 1994). The fourth report is from a VEP study with chronically implanted electrodes, which examined colour adaptation effect in epileptic patients (Allison *et al.* 1993). Visual stimuli in this study were a 1 s 'adaptation' checkerboard in colour, followed by a 100 ms 'test' checkerboard, either of the same colour as the adaptation stimulus ('same') or the different colour ('different'). Significant difference between test VEPs in the 'same' and 'different' conditions was found mostly in the posterior part of the fusiform and lingual gyri. Furthermore, when they applied electric stimulation to this region, the patient reported the sensation of shimmering lights with colour in the contralateral half-field. Taken together, these reports consistently indicate the functional specialization for colour perception in the posterior part of the fusiform gyrus.

According to anatomical studies by Clarke & Miklosy (1990), human V4 is a part of Brodmann's areas 19 and it is located within the fusiform gyrus (although they mentioned in their paper that the position of V4 was very approximate). Monkey V4 was first identified by Zeki (1971) based on cortical projections from V2 and V3. Subsequent studies of callosal connections (Zeki 1978) and retinotopic

Table 2. *PFGE activation with after-image versus without after-image*

(L, left; R, right. Talairach coordinates of ROI centres are stated as millimetres from the anterior end of the AC-PC line and correspond to the stereotactic conventions of the atlas of Talairach and Tournoux (Talairach & Tournoux 1988). PFGE response: condition (i)-(iii) is the difference between conditions (i) and (iii) for the time integral of the per cent change in MRI signal. This difference value was then multiplied by 100 and divided by the number of images for the following period: rest 1, the first resting period (image numbers 1-11); stim, the stimulation period (image numbers 11-26); rest 2, the second resting period (image numbers 26-41). After-image duration is stated as the number of seconds from the onset of the second resting period to the time when all after-images completely disappeared. Grand mean was calculated over all tested hemispheres, using mean data for subject 1.)

subject	side (L/R)	Talairach coordinates		PFGE response: condition (i)-(iii)			after-image duration in condition (i)
		x	y	rest 1	stim	rest 2	
1	L	-20	-64	0.7	15.6	114	41
1	L	-27	-60	-3.5	-94.7	62.3	48
	R	+22	-51	2.7	-37.2	116	—
Mean for 1	L	-24	-62	-1.4	-39.6	88.2	45
	R	+22	-51	2.7	-37.2	116	—
2	L	-23	-70	10.3	-51.3	83.2	50
3	R	+16	-65	6.8	-35.3	51.3	44
4	R	+13	-62	17.0	23.4	90.2	38
5	L	-9	-70	4.8	39.9	147	42
	R	+17	-60	-5.6	0.8	61.5	—
6	R	+14	-62	-4.7	35.0	57.0	37
grand mean		-19 (L) +16 (R)	-63	3.7	-8.0	87	43

mapping with multiunit recording (Gattass *et al.* 1988) enabled an accurate determination of the extent of V4. Considering the anatomical correspondence with the position of monkey V4, it is likely that human V4 falls just anterior to human VP (ventroposterior area of V3 complex). In fact, a recent fMRI study identified borders of multiple visual areas in human with phase-encoded retinotopical mapping, in which the vertical meridian is represented at the ventral VP-V4v (V4 ventral) border and the horizontal meridian is represented at the anterior border of ventral V4v (Serenio *et al.* 1995). The position of PFGE reported in our fMRI study is consistent with those of functionally proposed human V4v and anatomically proposed human V4, and all of them match the position of the human colour centre described above.

One of the difficulties in making animal models of achromatopsia is that little is known about critical processes in colour perception. Although colour discrimination tasks have been widely used to assess colour sense in both human and animal studies, there are other basic processes in colour vision, such as colour constancy and colour adaptation. It is notable that patients with achromatopsia are able to undertake some forms of isoluminant colour discrimination (Henaff & Michel 1993; Vaina 1994; Barbur *et al.* 1994), which may be comparable to a relatively mild impairment in hue discrimination caused by bilateral V4 lesions in monkeys (Heywood & Cowey 1987; Walsh *et al.* 1993). Gross impairment in isoluminant colour discrimination reported in patients with severe achromatopsia (Heywood *et al.* 1987, 1991) may be due to additional cortical lesions other than the fusiform gyrus. An earlier suggestion that monkey V4 is important for colour constancy (Zeki 1983), rather

than wavelength discrimination (Wild *et al.* 1985) has been recently confirmed, i.e. monkeys with V4 lesions showed deficits in performing previously overlearned colour discrimination tasks when the light components in the illuminant were changed (Walsh *et al.* 1993). Consistent with this evidence, one patient with achromatopsia showed clear impairment in colour constancy (Kennard *et al.* 1995). While colour adaptation deficits have not been demonstrated in achromatopsic patients, one lesion study in monkeys reported that V4 lesions produced a mild elevation in detection thresholds after adapting to colour stimuli (Schiller & Dolan 1994). To elucidate the underlying mechanisms of achromatopsia, well-designed testing methods for colour vision defects and functional measurement methods for identifying colour-processing areas have yet to be established. Because our study clearly demonstrates that fMRI can reveal colour perception even with after-image, it will be promising to use this technique further in establishing the functional specialization of human visual cortices.

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