NeuroImage

www.elsevier.com/locate/ynimg
NeuroImage 21 (2004) 1665–1673

The neural substrates of conscious color perception demonstrated using fMRI

Tomoyo Morita,[a] Takanori Kochiyama,[a] Tomohisa Okada,[b] Yoshiharu Yonekura,[c] Michikazu Matsumura,[a] and Norihiro Sadato[b,d,*]

[a] *Graduate School of Human and Environmental Studies, Kyoto University, Kyoto, Japan*
[b] *National Institute for Physiological Sciences, Okazaki, Japan*
[c] *Biomedical Imaging Research Center, Fukui Medical University, Fukui, Japan*
[d] *Japan Science and Technology Corporation (JST)/Research Institute of Science and Technology for Society (RISTEX), Kawaguchi, Japan*

Received 26 August 2003; revised 11 December 2003; accepted 11 December 2003

It is well established that seeing color activates the ventral occipital cortex, including the fusiform and lingual gyri, but less is known about whether the region directly relates to conscious color perception. We investigated the neural correlates of conscious color perception in the ventral occipital cortex. To vary conscious color perception with the stimuli-remaining constant, we took advantage of the McCollough effect, an illusory color effect that is contingent on the orientation of grating stimuli. Subjects were exposed to a specific combination of chromatic grating patterns for 10 min to induce the McCollough effect. We compared brain activities measured while the subjects viewed achromatic grating stimuli before (PRE) and after the induction of the McCollough effect (POST) using functional magnetic resonance imaging (fMRI). There were two groups: one group was informed that they would perceive illusory color during the session (INFORMED group), whereas the other group was not informed (UNINFORMED group). The successful induction of the McCollough effect was confirmed in all subjects after the fMRI experiment; nevertheless, only approximately half of the UNINFORMED subjects had been aware of the color during the POST session, while the other half had not. The left anterior portion of the color-selective area in the ventral occipital cortex, presumably V4α, was significantly active in subjects who had consciously perceived the color during MR scan. This study demonstrates the activity in a subregion of the color-selective area in the ventral occipital cortex directly related to conscious color perception.

Keywords: McCollough effect; Illusory aftereffect; Color perception; Awareness; V4 cortex; Functional MRI

Introduction

Subjective color experience is primarily elicited by the neuronal processing of the wavelength composition of the light that reaches the eyes. Electrophysiological studies in nonhuman primates have revealed that chromatic interaction was found at multiple levels from LGN (Kastner et al., 1992), V1 (Wachtler et al., 2003), V4 (Zeki, 1983), and inferior temporal cortex (Komatsu et al., 1992). Visual areas V1/V2 are primarily involved in the earlier stages of color processing, in which the presence and the intensity of different types of wavelengths are registered, whereas responses of cells in V1 are mildly modulated by the surrounding wavelength composition (Wachtler et al., 2003). In the higher order visual areas V4, however, cells are shown to respond to colored patches based on what they are perceived, regardless of the wavelength composition of the patches (Zeki et al., 1983). These results suggest that the activities in the higher order visual cortex reflect the perception more strongly than the early visual cortex.

Human neuroimaging studies have demonstrated that passive viewing of colored stimuli and simple color discrimination activate the fusiform and lingual gyri (Corbetta et al., 1991; Gulyas and Roland, 1994; McKeefry and Zeki, 1997). Using multicolored abstract or natural scenes whose wavelength composition or illumination intensity changes continually, without altering the perceived color of the individual patches, Bartels and Zeki (2000) found two active zones in the fusiform gyrus, V4 proper and V4α. Because of the coactivation by the color constancy tasks, they grouped them as V4 complex, designating it as the color center of the human brain. Detailed retinotopic study using functional MRI and phase encoding technique by Wade et al. (2002) revealed that the color center includes the fourth visual field (hV4) that is anterior to the V3v: they observed that the color stimulation activated the V1, V2, hV4, and further anterior ventral occipital region.

Recent studies have shown that illusory color effects, which can elicit color perception in the absence of chromatic stimuli, also activate a portion of the color center (Barnes et al., 1999; Hadjikhani et al., 1998; Sakai et al., 1995). This suggests that the activity in the color center may be necessary for color perception, which is consistent with lesion studies showing that achromatopsic human patients lose color perception following damage to fusiform and lingual gyri (Damasio et al., 1980; Meadows, 1974; Zeki, 1990). Less is known, however, about

* Corresponding author. Section of Cerebral Integration, Department of Cerebral Research, National Institute for Physiological Sciences, Myodaiji, Okazaki, Aichi, 444-8585, Japan. Fax: +81-564-55-7786.
E-mail address: sadato@nips.ac.jp (N. Sadato).
Available online on ScienceDirect (www.sciencedirect.com.)

1053-8119/$ - see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.neuroimage.2003.12.019

how activity in the color center relates to conscious color perception.

To examine how activity in the color center relates to conscious color perception, we utilized an illusory color effect called the McCollough effect (McCollough, 1965), in which the intensity of color perception varies while the stimuli remain constant. To induce the McCollough effect, two orthogonally oriented grating patterns, such as a green-and-black horizontal grating and a magenta-and-black vertical grating, are viewed alternatively for a few minutes (Fig. 1c). After such induction, black-and-white test patterns are presented to subjects. White section of the horizontal grating appears to the viewer to be tinged pink, whereas that of the vertical grating appears green, complementary to the color of the induction stimuli. It is known that the McCollough effect is an automatically and inevitably induced phenomenon.

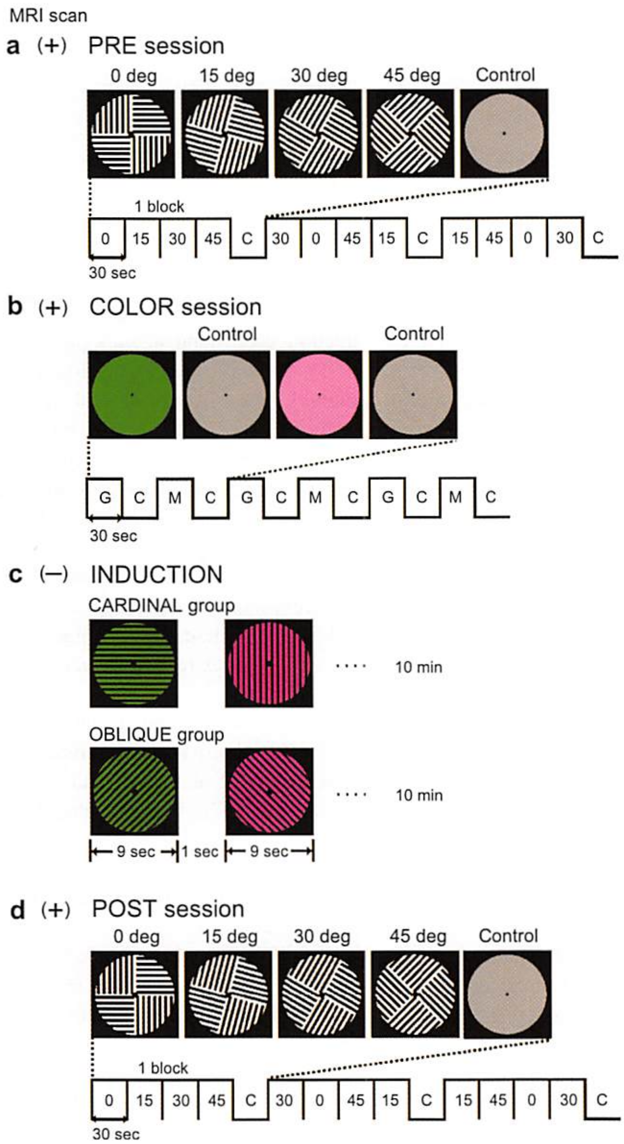


Fig. 1. Experimental procedure and stimuli. In the PRE (a) and POST (d) sessions, subjects viewed gratings rotated 0°, 15°, 30°, and 45° and a gray, control (C) pattern for 30 s each in each block. In the COLOR (b) session, green (G) and magenta (M) circular stimuli were presented alternately for 30 s each, interleaved with a gray, control pattern. During the INDUCTION (c) phase, subjects in the CARDINAL group were presented with green horizontal grating and magenta vertical grating. Subjects in the OBLIQUE group were presented with the same stimuli rotated counterclockwise 45°. fMRI images were acquired during sessions (a), (b), and (d), but not during (c).

As the McCollough effect can induce color perception without chromatic stimuli, it is well suited for the investigation of the conscious color perception. Our hypothesis was that the induction of the McCollough effect and conscious illusory color perception are different processes and hence neural representations are different. If this hypothesis is true, there may be subjects who are not consciously aware of the illusory color whereas the McCollough effect has been induced. To prove this hypothesis, we examined subjects who were not provided a priori information of the McCollough effect (UNINFORMED group). Neural activities were measured before and after the induction of the McCollough effect using functional magnetic resonance imaging (fMRI). Half of the subjects did not perceive illusory color during fMRI session after the induction (UNAWARE group) and others did (AWARE group), whereas psychophysical test after fMRI confirmed the induction of the McCollough effect in all subjects. Then we recruited another group who was informed that they would perceive illusory color during the session and was required to pay attention to the color (INFORMED group). All subjects in the INFORMED group perceived illusory color during the fMRI session. Common activation across all groups was in the posterior portion of the color center on the left, presumably V4, which may represent the induction of the McCollough effect. On the other hand, common activation between AWARE and INFORMED groups, but not in the UNAWARE group, was found in the anterior portion of the color center, presumably V4α, on the left. Hence, the left V4α may represent the perception of the illusory color.

Methods

Subjects

A total of 14 men and 7 women (mean age: 23.0 ± 2.3 years) participated. All subjects had normal color vision, confirmed using Ishihara plates (Ishihara, 1971). None of them had any prior knowledge of the McCollough effect. Fifteen subjects (10 men and 5 women, mean age: 23.2 ± 2.4 years) were not informed and they were instructed to passively view the stimuli presented (UNINFORMED group). On the other hand, before the scanning, six subjects (four men and two women, mean age: 22.3 ± 1.9 years) were explicitly informed that they may perceive the illusory color, and they attended to the illusory color during the POST session (INFORMED group). The Ethical Committee of Fukui Medical University approved the study protocol, and written informed consent was obtained from each volunteer before participation.

Experimental design

The subjects lay in the fMRI scanner, their heads were immobilized with an elastic band and sponge cushions, and their ears were plugged. The room lights were turned off. Visual stimuli were presented on a rear projection screen placed at the foot of the scanner bed and viewed by the subjects through a mirror mounted on the head coil. All stimuli were circular, subtended a visual angle of 8.8°, and had a central fixation spot with a diameter of

0.18°. The subjects were instructed to continue viewing the central fixation point throughout the sessions. The experiment consisted of three fMRI scanning sessions: first a PRE session, followed by a COLOR session, and finally a POST session. Between the COLOR and POST sessions, stimuli for the induction of the McCollough effect were presented with no scanning (Fig. 1). The PRE session contained three blocks, each of which consisted of four grating epochs (0°, 15°, 30°, and 45°). The order of the orientation of grating presented was pseudo-randomized, followed by a control epoch. In the 0° epoch, the stimuli presented were a combination of vertical and horizontal square-wave gratings (with a spatial frequency of 3.5 cycles/degree and a mean luminance of 22.12 cd·m^{-2}) assigned alternately to the divided quadrants in a circular 8.8° window (Fig. 1). The stimuli presented for the 15°, 30°, and 45° epochs were the same as the 0° epoch with the grating rotated clockwise in 15° steps. Each epoch lasted for 30 s. In the control epoch, subjects viewed a uniform gray stimulus [Commission International d'Eclairage (CIE) x and y coordinates: 0.276, 0.300] with the same size and luminance as the grating stimuli.

In the COLOR session, a uniform color stimulus was presented to identify color-specific areas of cortex. In this session, green (CIE x and y coordinates: 0.286, 0.584) and magenta (0.276, 0.208) circular stimuli were presented in alternating blocks of 30 s each, interleaved with control blocks of 30 s each. A total time series consisted of 12 epochs. The control stimulus was the same as the one used in the PRE session. The color stimuli had the same luminance as the control stimulus (Fig. 1).

Following the COLOR session, subjects were exposed to a color aftereffect induction procedure: a specific combination of orientation and color components to induce the McCollough effect. This session was conducted in the scanner without acquiring fMRI data. Eleven subjects (eight UNINFORMED, three INFORMED) were alternately exposed to two chromatic gratings with a spatial frequency of 3.5 cycles/degree, for a total of 10 min: one was green (CIE x and y coordinates: 0.284, 0.592) with a horizontal black grating and the other was magenta (0.275, 0.145) with a vertical black grating oriented orthogonal to the preceding horizontal grating. Another group of 10 subjects (7 UNINFORMED, 3 INFORMED) was exposed to the same stimuli rotated counter-clockwise by 45°. The former group comprised the CARDINAL group and the latter group was the OBLIQUE group. Each stimulus was presented for 9 s with a 1-s interstimulus interval. During the interstimulus interval, subjects were required to perform a simple calculation task. In this task, two numerals were presented for 500 ms in the center of the screen, followed by a black screen for 500 ms. The subjects indicated whether the sum of these two numerals was odd or even by raising their right thumb to indicate odd sums. This was done to keep the subjects awake. The POST session, which followed the induction procedure, was performed exactly as the PRE session. The order of presentation of the four gratings was randomized.

Functional MR image acquisition

Imaging data were acquired using T2*-weighted, gradient echo-planar sequences using 3T MRI systems (SIGNA-LX, GE, Milwaukee, USA). A total of 157 images were acquired during the PRE and POST sessions, and 127 images were acquired during the COLOR session. The time interval between two successive acquisitions of the same slice was 3000 ms, with an echo time of 30 ms and a flip angle of 90°. Each volume consisted of 36 slices with a slice thickness of 3.5 mm, with a 0.5-mm gap, to cover the entire brain. The field of view was 22 × 22 cm, and the matrix size was 64 × 64, giving voxel dimensions of x, y, z = 3.44 × 3.44 × 4 mm.

Immediately after the POST session, when the subjects were still lying in the scanner, they were asked whether they had experienced the illusory color during the POST session. After this, a T2-weighted anatomical image was obtained using a fast spin echo sequence with location variables identical to those of the EPI images. The repetition time was 5000 ms, with an echo time of 67 ms and an FOV of 22 cm. The in-plane matrix size was 256 × 256 pixels, with a pixel size of 0.859 × 0.859 mm. In addition, high-resolution whole-brain MR images were obtained using a conventional T2-weighted, fast spin echo sequence with a flip angle of 90°, echo time of 67 ms, repetition time of 6000 ms, and FOV of 22 cm. A total of 112 transaxial images were obtained. The in-plane matrix size was 256 × 256, slice thickness was 1.5 mm, and pixel size was 0.859 × 0.859 mm.

Evaluation of the McCollough effect

The McCollough effect for each subject was evaluated by a report of the intensity of the illusory color perceived by each subject. After completion of the fMRI procedures, subjects were taken out of the scanner and viewed the same four achromatic gratings printed on paper one by one as presented in the PRE and POST sessions, positioned such that they had the same visual angle as in the scanner. They rated the illusory color intensity by selecting colors from 21 color samples that best matched those they perceived on each test stimulus with each orientation (0°, 15°, 30°, and 45°). Ten of these samples were green, with a fixed hue and value but with increasing saturation, ranging from G1 (CIE x and y coordinates: 0.275, 0.296) to G10 (0.275, 0.348). The other 10 were magenta ranging from M1 (0.273, 0.291) to M10 (0.273, 0.253). The subjects, who reported that they did not perceive the illusory color during the POST session, became aware of the illusory color during the McCollough effect evaluation.

Data analysis

Data were analyzed using Statistical Parametric Mapping (SPM99; Wellcome Department of Cognitive Neurology, London, UK) (Friston et al., 1995a). Images were realigned using the last image as a reference. Then, the T2-weighted anatomical images scanned in planes identical to the functional imaging slice were coregistered to the first scan in the functional images. Following this, the coregistered T2-weighted anatomical image was normalized to a standard T2 template image supplied with SPM99. This template image closely approximates the space described in the atlas of Talairach and Tournoux (1988). The parameters from this normalization process were then applied to each of the functional image. Finally, these spatial normalized functional images were smoothed with an isotopic Gaussian kernel 6 mm to compensate for the anatomical variability between subjects. The high-resolution anatomical image was also normalized as the same procedure.

In single-subject analysis, increased neural activity was identified by the general linear model with temporally correlated errors (Friston et al., 1994, 1995a,b; Worsley and Friston, 1995). The time series data in the PRE and the POST session were entered into a single-design matrix, which is composed of 2 × 4 different regressors for each orientation of each session (PRE, 0°;

PRE, 15°; PRE, 30°; PRE, 45°; POST, 0°; POST, 15°; POST, 30°; POST, 45°) and two constant terms (PRE and POST session effects). Each regressor was constructed with the stimulus locked box-car functions convolved with the hemodynamic function. For COLOR session, another design matrix was specified as the same way. Global signal changes were removed by scaling. Band pass filter, which was composed of the discrete cosine basis function with a cut-off period of 120s for high pass filter and a canonical hemodynamic response function for low pass filter were applied to improve signal to noise ratio. In each subject, appropriate contrast images to evaluate the activity related to the visual processing of grating (PRE with pooling all gratings vs. control) and actual color stimuli (COLOR vs. control), and the activity related to the McCollough effect and illusory color perception (POST vs. PRE, where all gratings were pooled in each session) were also constructed for the following random-effects analysis.

A random-effects model was used to make statistical inferences at the population level (Friston et al., 1999). One sample t test was performed using the contrast images specified above. A height statistical threshold of $P < 0.001$ with an extent threshold of $P < 0.05$ corrected for multiple comparison was applied. In the volume of interest analysis, the time course of the data was converted to a percent signal change, which was a value of percent change in the mean adjusted signals during each orientation condition relative to the control (gray) conditions, excluding the first two scans during the transition phase of each epoch. These data were used to investigate the relation between the brain activity response to the grating with each orientation (0°, 15°, 30°, 45°) in the POST session, and the intensity of the illusory colors elicited by the same grating.

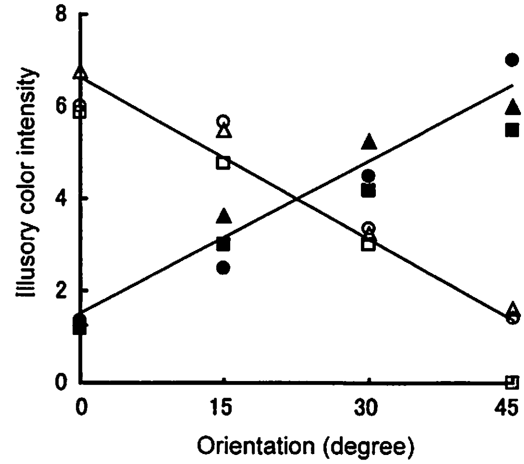


Fig. 2. The McCollough effect was induced in all subjects. The averaged intensity of the illusory color elicited by the grating with each orientation (0°, 15°, 30°, and 45°) in the CARDINAL (open symbols) and OBLIQUE (closed symbols) groups, which was evaluated after the POST session. The CARDINAL group consisted of subjects from the AWARE (△; n = 4), UNAWARE (□; n = 4), and INFORMED (○; n = 3) groups. Similarly, the OBLIQUE group consisted of subjects from the AWARE (▲; n = 4), UNAWARE (■; n = 3), and INFORMED (●; n = 3) groups.

Results

Induction of the McCollough effect

We asked the subjects directly after the completion of the fMRI scanning if they had perceived the illusory color during the fMRI scanning session after the induction of the McCollough effect (POST session). All subjects in the INFORMED group reported that they had perceived the illusory color. In contrast, only eight subjects in the UNINFORMED group reported that they had perceived the illusory color (AWARE group), while seven subjects reported that they had not (UNAWARE group). Nevertheless, the McCollough effect was successfully induced in all subjects, confirmed by an evaluation performed after all MRI sessions were completed (Fig. 2). Because the subjects perceived illusory green and magenta concurrently, each tingeing the corresponding grating, the magnitudes of the illusory green and magenta were averaged within each subgroup for each orientation. The intensity of the illusory color was tuned to the orientation of the induction stimuli, confirming the presence of the McCollough effect. The three CARDINAL subgroups exhibited the strongest illusory effect with 0° rotation (the same orientation as the induction stimuli), which gradually decreased as the orientation of the test stimuli deviated from the induction stimuli. The OBLIQUE subgroups exhibited the reverse pattern with the maximum illusory effect with 45° rotation. A three-way ANOVA [induction stimuli (CARDINAL, OBLIQUE; two) × test stimuli (0°, 15°, 30°, and 45°; four) × subgroups (AWARE, UNAWARE, and INFORMED; three)] confirmed the significance of the interaction [$F(3,12) = 49.90$, $P < 0.001$]. There was no significant group effect [$F(2,14) = 0.18$]. The CARDINAL and OBLIQUE groups exhibited the same performance except for the orientation to which the McCollough effect was tuned. Thus, subjects in both groups were pooled in the following analyses.

Identification of color-selective areas

Using fMRI, we identified color-selective areas using actual chromatic stimulation (Fig. 1b). Compared with gray stimuli, chromatic stimuli strongly activated the ventral visual pathway, including V1/V2, and portions of the fusiform and lingual gyri. Activity in the fusiform and lingual areas was observed bilaterally and extended the anterior/posterior length, concordant with previous reports of V4 complex (Bartels and Zeki, 2000; Beauchamp et al., 1999). We will call this area as V4 in the following text.

Within the areas involved in the processing of actual color stimuli, we identified regions also active during the perception of illusory color. All subjects in the AWARE and INFORMED groups perceived illusory color during the POST session. To determine activity related to both the induction of the McCollough effect and illusory color perception, one sample t test was performed using the POST versus PRE (pooling all gratings) contrast images of these subjects. This comparison revealed that there was significant bilateral activity in the fusiform gyri (Fig. 3b). The activated area in the left hemisphere extended the full anterior/posterior length of V4, peaking at (−28, −80, −16) and (−36, −56, −14). These peaks fit well (Table 1) with the two anatomically delineated V4 subregions, designated V4 (posterior) and V4α (anterior), respectively (Bartels and Zeki, 2000). The size in the right hemisphere was smaller than that on the left, peaking at the coordinates (34, −66, −18). We set the region of interest around each peak, posterior LV4 at (−28, −80, −16),

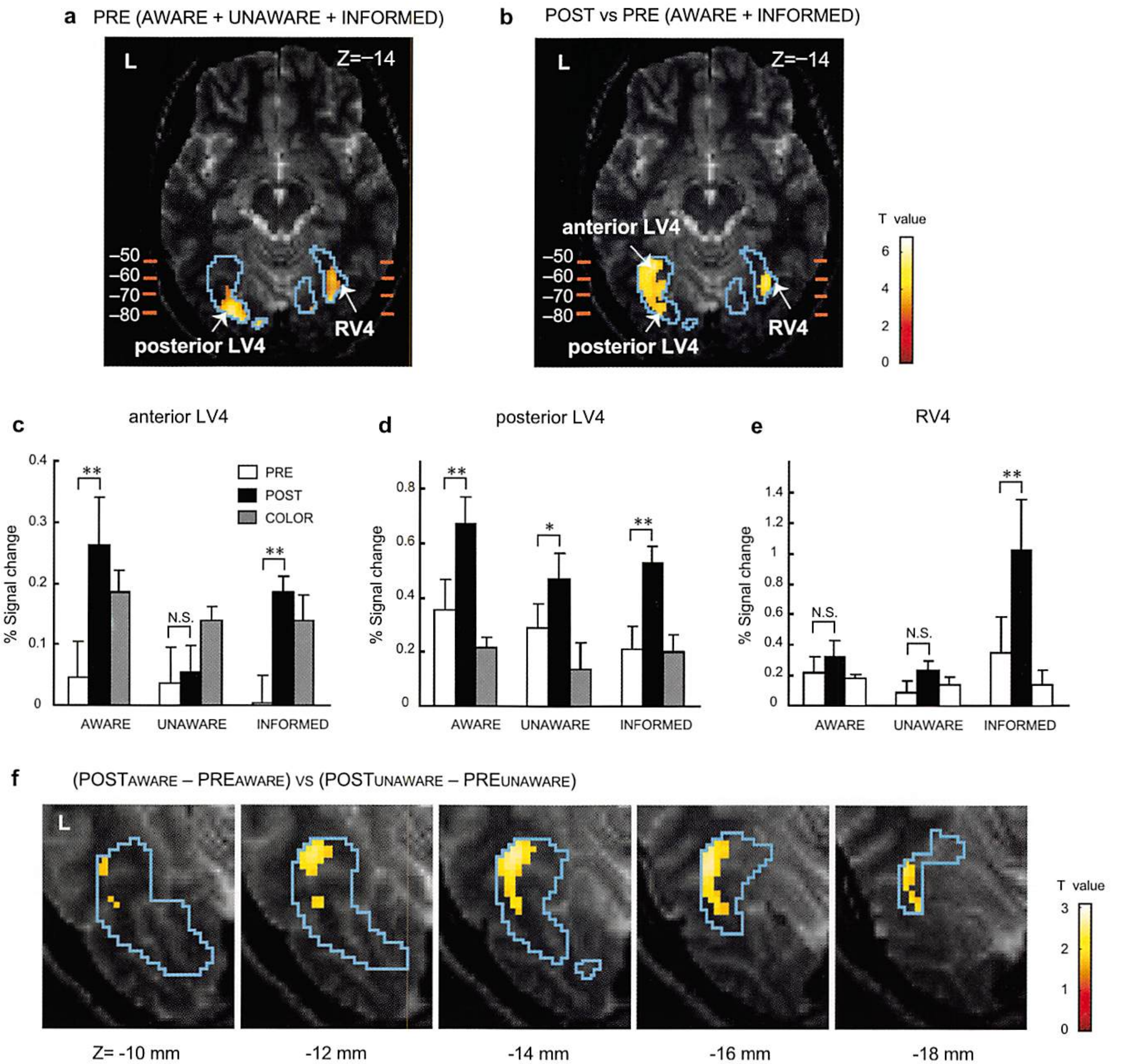


Fig. 3. Functional subdivision of the color-selective V4. The light-blue outlines indicate areas significantly activated by actual chromatic stimuli compared with gray stimuli by group analysis including all subjects. (a) The brain areas exhibiting significantly increased activity to the achromatic grating. These were defined by comparing the response to gratings (0°, 15°, 30°, and 45°) versus gray (control) during the PRE session in all subjects (AWARE + UNAWARE + INFORMED). An SPM{T} was superimposed on a representative T2-weighted image. Orange scales show the coronal plane $Y = -50$ to $Y = -80$. The statistical threshold was set at $P < 0.001$ uncorrected for height threshold, and $P < 0.05$ corrected for cluster level. The activated area was observed bilaterally in the posterior V4. (b) The brain areas exhibiting significant session differences (POST−PRE) in task-related activity (gratings−gray) in subjects who perceived the illusory color during the POST session (AWARE + INFORMED). The same format and scale as in (a). A cluster in the left fusiform gyrus exhibited two separate local peaks: one in the anterior LV4 and the other in the posterior LV4. (c–e) Bar graphs show the mean percent signal change of these peaks. Error bars represent the standard error of the mean. $*P < 0.01$, $**P < 0.001$. (c) The anterior LV4 was significantly more active during the POST session compared with the PRE session in the AWARE and INFORMED groups, but not in the UNAWARE group. (d) The posterior LV4 was significantly active during the PRE session, and more active during the POST session compared with the PRE session in each group. (e) The RV4 was significantly more active during the POST session compared with the PRE session only in the INFORMED group. (f) Transverse sections illustrating that the anterior LV4 of the AWARE is more active than UNAWARE group during the POST session than the PRE session. An SPM{T} was superimposed on a representative T2-weighted image. A threshold of $T > 1.73$ was used for display purpose.

anterior LV4 at (−36, −56, −14), and RV4 at (34, −66, −18). The percent changes in activity were calculated at each peak for each group and each session (Figs. 3c, d, e).

Fig. 4 shows typical results from AWARE group demonstrating significant increased activity during the POST session than PRE ($P < 0.01$ uncorrected). There were two separated clusters in the

Table 1
Brain regions more active after the induction procedure than before (POST-PRE) in the AWARE and INFORMED groups

Brain region	Cluster size (voxels)	Coordinate			T value
		x	y	z	
LV4	251*	−36	−56	−14	5.56
		−28	−80	−16	5.18
RV4	58*	34	−66	−18	6.65

*$P < 0.05$ corrected for multiple comparisons at the cluster level.

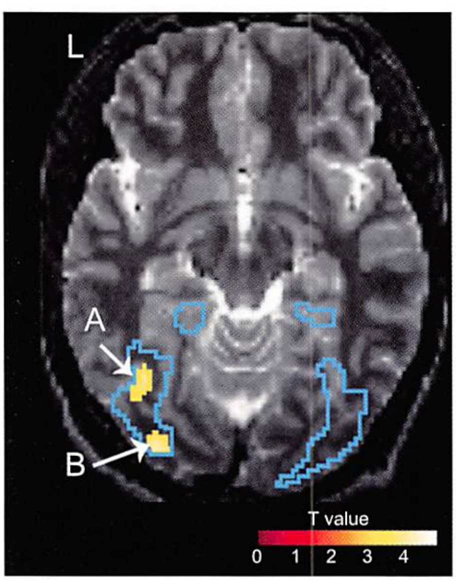
left color-selective area, which was consistent with the result of random-effect analysis.

Anterior LV4

The anterior LV4 was active during the POST session in the AWARE and INFORMED groups, but not in the UNAWARE group. Furthermore, the anterior LV4 was not active during the PRE session in any group. A two-way ANOVA with session (PRE and POST) and group (AWARE, UNAWARE, and INFORMED) with repeated measures revealed a significant session effect [$F(1,18) = 31.41$, $P < 0.001$], and a significant interaction between group and session [$F(2,18) = 6.331$, $P < 0.01$] (Fig. 3c). These results suggest that in addition to actual color processing, activity in the anterior LV4 was related to conscious illusory color perception. We also performed a multiple linear regression analysis to examine the relation between the neural activity in the anterior LV4 and the intensity of the illusory color perceived. In the AWARE group, the activity increased linearly as the mean intensity of the illusory color increased [$F(1,23) = 8.678$, $P < 0.01$] (Fig. 5). In the INFORMED group, however, there was no significant relation between the neural activity level and the intensity of the illusory color perceived [$F(1,17) = 0.124$].

Posterior LV4

The posterior LV4 was active during the PRE session, indicating that the posterior LV4 was involved in processing information from both the grating and color stimuli (Fig. 3a).



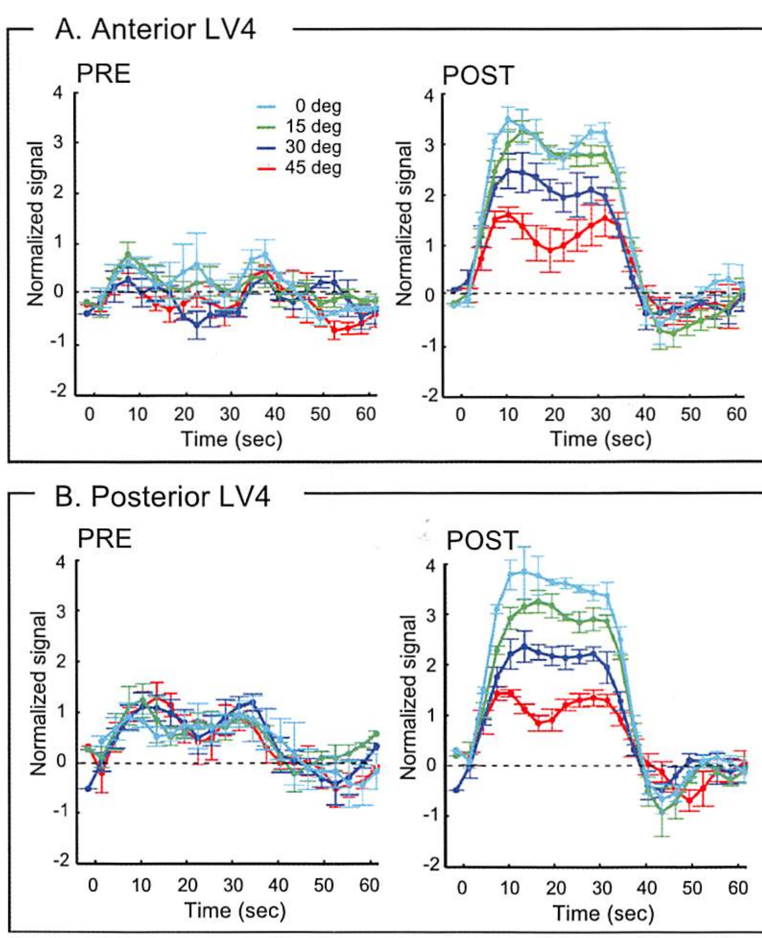


Fig. 4. Left: Regions with significant increased activity during the POST session compared with the PRE session in a single subject from the AWARE group, who belongs to the CARDINAL group. A statistical parametric map was overlaid on the subject's high resolution MRI that was normalized to the Talairach's standardized space (axial image, $z = -12$ mm). The statistical threshold was set at $P < 0.01$ uncorrected. There were two discrete clusters in the left ventral occipital regions: the anterior LV4 (A) and the posterior LV4 (B). The light blue outlines indicate areas significantly activated by actual chromatic stimuli compared with gray stimuli. Right: Mean time series from the ROI of the anterior LV4 (A) and the posterior LV4 (B) averaged over three epochs of each orientation. Error bars represent the standard error of the mean. A vertical axis represents the normalized fMRI signal. Only the posterior LV4 was active during PRE session, while both of the anterior and the posterior LV4 showed activity depending on the orientation during the POST session.

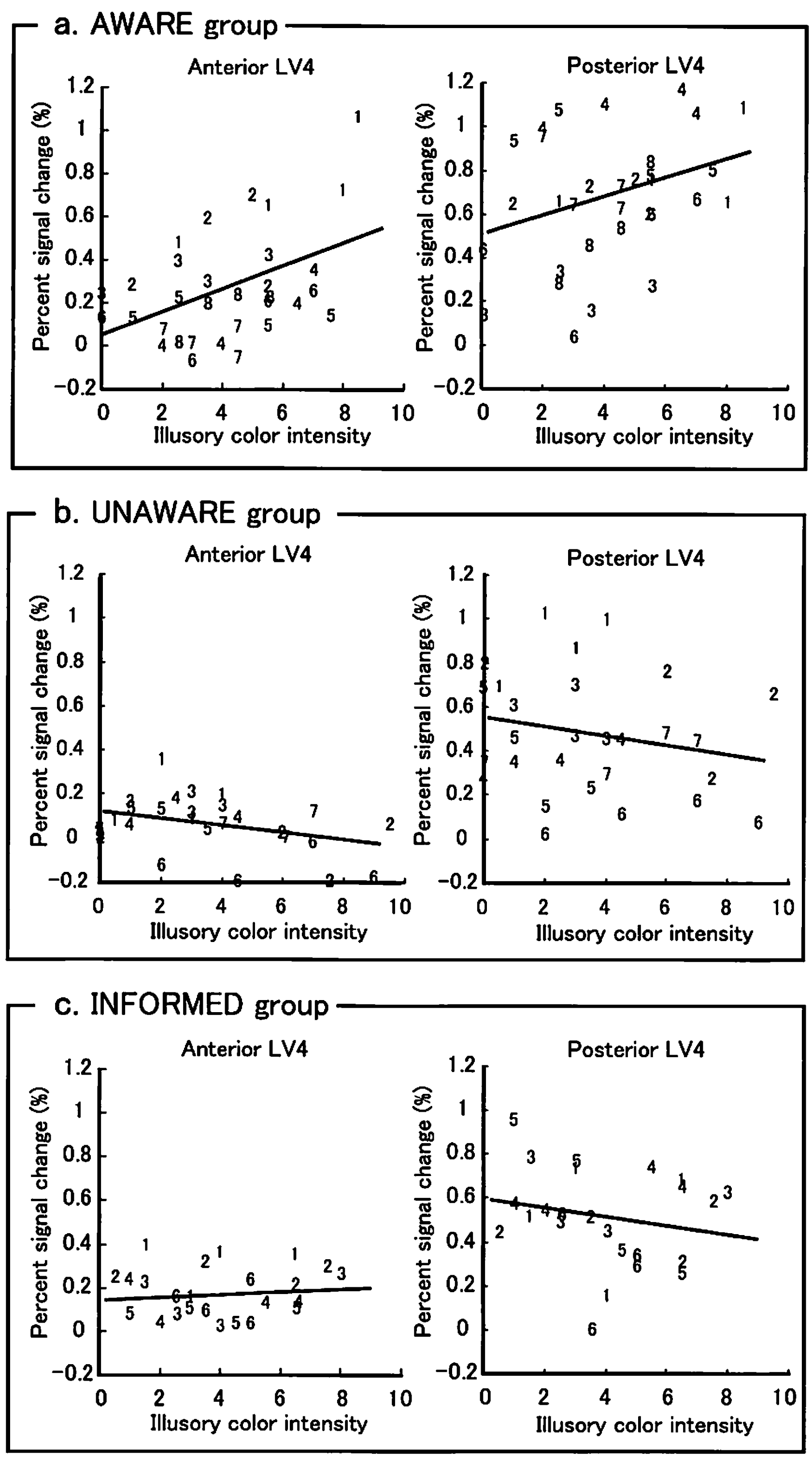


Fig. 5. Neural activities correlated with illusory color intensity. The graphs show the relationship between the percent signal change in the anterior LV4 and the posterior LV4 for each orientation condition (0°, 15°, 30°, 45°) during the POST session, and the intensity of the illusory colors elicited by the same orientation, which were evaluated after the POST session. Thus, each subject yielded four data points that are represented by the same numeral for each subject in each graph. (a) Multiple regression analysis including the intensity of the illusory colors and a dummy variable for subject revealed a significant regression of the neural activity in the anterior LV4, on the intensity of illusory colors in the AWARE group [$F(1,23) = 8.678$, $P < 0.01$] (a), and a trend was found in the posterior LV4 [($F(1,23) = 2.045$, $P = 0.167$). (b) In the UNAWARE group, there was no significant correlation between the neural activity level and the intensity of the illusory color perceived in either subregion of the LV4. (c) In the INFORMED group, there was no significant correlation between the neural activity level and the intensity of the illusory color perceived in either subregion of the LV4.

Furthermore, the posterior LV4 was more active during the POST session compared to the PRE session in all groups. There was a significant main effect of session [$F(1,18) = 37.12$, $P < 0.001$], but there was no significant group effect [$F(2,18) = 0.918$] or interaction between group and session [$F(2,18) = 1.075$] (Fig. 3d). This finding indicates that the posterior LV4 was involved in the induction of the McCollough effect. Furthermore, there is no significant correlation between the neural activity in the posterior LV4 and the intensity of the illusory color perceived in the UNAWARE and INFORMED group, but a trend was found in the AWARE group [AWARE: $F(1,23) = 2.045$ ($P = 0.167$); UNAWARE: $F(1,20) = 0.213$; INFORMED: $F(1,17) = 1.148$].

Right V4

The coordinates and the pattern of activity in the RV4 were different from either the anterior or posterior LV4. Only in the INFORMED group was the RV4 more active during the POST session compared to the PRE session. An ANOVA revealed a significant main effect of session [$F(1,18) = 27.51$, $P < 0.001$], and there was also a significant interaction between group and session [$F(2,18) = 9.176$, $P < 0.01$] (Fig. 3e). This result suggests that the RV4 was strongly active in subjects who were attending to color perception during the POST session. Furthermore, there is no significant correlation between the neural activity in the RV4 and the intensity of the illusory color perceived in the UNAWARE and INFORMED group, but a trend was found in the AWARE group [AWARE: $F(1,23) = 2.975$ ($P = 0.098$); UNAWARE: $F(1,20) = 2.013$; INFORMED: $F(1,17) = 0.559$].

Discussion

The results of the present study show that different portions of human V4 were active during different types/stages of color stimuli processing and perception. Activity in the posterior LV4 appeared to be related to the processing of chromatic stimulation and the induction of the McCollough effect, while activity in the anterior LV4 appeared to be related to conscious color perception. Activity in the RV4 may be strongly modulated by attention to color.

Illusory color perception

Of the UNINFORMED group (those without prior information), approximately half of the subjects noticed the illusory color during the POST session, whereas the others did not. Nevertheless, in the evaluation immediately after all MR images were acquired, when we instructed the subjects to be aware of the illusory color, the subjects who had not previously noticed the illusion, then also perceived the illusory color. Furthermore, the evaluation confirmed that the McCollough effect was successfully induced in all subjects and was similar across the subjects. The McCollough effect has been shown to be induced immediately upon priming, peak 1 min after priming, and decline very gradually afterwards (Riggs et al., 1974); therefore, the McCollough effect should have been induced by the time of the POST session in all subjects. This dissociation of the induction of the McCollough effect and the perception of the illusory color suggest that separate neural substrates underlie these processes.

Color-selective area

The color-selective area was observed in the bilateral fusiform gyrus, as delineated by activation in response to chromatic stimuli. The activity extended anteroposteriorly along the collateral sulcus, which is probably consistent with the V4 complex as defined previously by Bartels and Zeki (2000). V4 itself is topographically organized, while V4α is not (Bartels and Zeki, 2000). Wade et al. (2002) have described that hV4 contains a hemifield representation, and a more anterior region contains a distinct foveal representation, which probably correspond to the Zeki's V4 and V4α, respectively.

UNINFORMED group (AWARE and UNAWARE groups)

Within the color-selective V4, the posterior LV4 was also activated by achromatic grating during the PRE session. Thus, the posterior LV4 was sensitive to both color and achromatic grating. In this area, the achromatic task-related activity was higher during the POST session compared to the PRE session in both groups, even in the UNAWARE group who was not aware of the illusory color. As the McCollough effect was also successfully induced in this group, the enhanced response in the posterior LV4 may reflect the linkage between the achromatic gratings to specific colors, but not the conscious color perception.

The anterior LV4, on the other hand, was activated by chromatic stimuli, but not by the achromatic grating, confirming that it is a color-specific area. Furthermore, during the POST session, the anterior LV4 was active only in subjects of the AWARE group, who were aware of the illusory color during the POST session, and not in those who were not aware of it. Hence, the anterior LV4 may be involved in conscious color perception evoked either by actual chromatic stimuli or an illusory effect. This functional segregation in V4 area may support recent imaging study indicating that the active use of color information recruits color-selective areas, particularly anterior fusiform gyrus, more extensively than passive viewing (Beauchamp et al., 1999).

INFORMED group

The INFORMED group showed activation in the both posterior and anterior LV4 as the AWARE group did. In AWARE group, however, there was a significant correlation between the intensity of the illusory color and the neural activity in the anterior LV4, whereas not in INFORMED group. Furthermore, the RV4 was strongly active during the POST session compared to the PRE session in INFORMED group, but not in AWARE group. These differences may be related to the top-down attentional effect, because selective attention to color modulates the V4 responses (Chawla et al., 1999; Corbetta et al., 1991; Spitzer et al., 1988). Subjects in the INFORMED group were required to pay attention to the expected color attributes that may be observed, while those in the AWARE group might allocate bottom-up sensory-driven attention to the color.

The right lateralized activation of V4 observed in the INFORMED group may be consistent with previous reports. Kosslyn et al. (2000) reported that instruction to attend to color causes increased activity in the right, but not the left fusiform area, regardless of stimulus, either color or gray scale. Moreover, Howard et al. (1998) has shown that color imagery activates the right fusiform gyrus, but not the left. The right fusiform area appears to be modulated by a top-down attentional system that is independent of the actual stimuli.

Laterality of the subdivisions in V4

In the present study, functional subdivisions in the V4 complex were observed only in the left hemisphere, although activity in response to actual color stimuli extended toward the anterior V4 in both hemispheres. The activation pattern of the RV4 was different from the LV4. The activity in the RV4 was not significantly related to the task, as shown by the POST–PRE session comparison, in either the UNAWARE or AWARE group. Thus, the RV4 did not appear to be involved in the induction of the McCollough effect or the perception of illusory color, suggesting the lateralization of the function of area V4. A few studies have demonstrated that color perception is lateralized to the left hemisphere in ventral regions with real color stimuli (Beauchamp et al., 1999; Gulyas and Roland, 1994) and without them (Barnes et al., 1999; Nunn et al., 2002). Further work is clearly necessary to confirm that conscious color perception is also lateralized to the left hemisphere.

Taken together, the results of the present study support the idea that conscious actual and illusory color perception occurs as a separate process in a discrete area of the fusiform gyrus, presumably V4α on the left. Processing of the physical composition of light stimuli and the induction of the McCollough effect occurs in the left V4 proper. These findings confirm the functional segregation of the color-selective areas of human visual cortex.

Acknowledgments

The authors thank the subjects who participated in this study and the staffs of the Biomedical Imaging Research Center and the Department of Radiology of Fukui Medical University for their assistance with data collection. This study was supported in part by a research grant for the "Research for the Future" Program (JSPS-RFTF97L00203) and a Grant-in-Aid for Scientific Research (B)(1) 14380370 from the Japan Society for the Promotion of Science.

References

Barnes, J., Howard, R.J., Senior, C., Brammer, M., Bullmore, E.T., Simmons, A., David, A.S., 1999. The functional anatomy of the McCollough contingent colour after-effect. NeuroReport 10 (1), 195–199.

Bartels, A., Zeki, S., 2000. The architecture of the colour centre in the human visual brain: new results and a review. Eur. J. Neurosci. 12 (1), 172–193.

Beauchamp, M.S., Haxby, J.V., Jennings, J.E., DeYoe, E.A., 1999. An fMRI version of the Farnsworth-Munsell 100-Hue test reveals multiple color-selective areas in human ventral occipitotemporal cortex. Cereb. Cortex 9 (3), 257–263.

Chawla, D., Rees, G., Friston, K.J., 1999. The physiological basis of attentional modulation in extrastriate visual areas. Nat. Neurosci. 2 (7), 671–676.

Corbetta, M., Miezin, F.M., Dobmeyer, S., Shulman, G.L., Petersen, S.E., 1991. Selective and divided attention during visual discriminations of shape, color, and speed: functional anatomy by positron emission tomography. J. Neurosci. 11 (8), 2383–2402.

Damasio, A., Yamada, T., Damasio, H., Corbett, J., McKee, J., 1980. Central achromatopsia: behavioral, anatomic, and physiologic aspects. Neurology 30 (10), 1064–1071.

Friston, K.J., Jezzard, P., Turner, R., 1994. Analysis of fMRI time-series. Hum. Brain Mapp. 1, 153–171.

Friston, K.J., Holmes, A.P., Poline, J.B., Grasby, P.J., Williams, S.C., Frackowiak, R.S., Turner, R., 1995a. Analysis of fMRI time-series revisited. NeuroImage 2 (1), 45–53.

Friston, K.J., Holmes, A.P., Worsley, K.J., Poline, J.B., Frith, C.D., Frackowiak, R.S., 1995b. Statistical parametric maps in functional imaging: a general linear approach. Hum. Brain Mapp. 1, 153–171.

Friston, K.J., Holmes, A.P., Price, C.J., Buchel, C., Worsley, K.J., 1999. Multisubject fMRI studies and conjunction analyses. NeuroImage 10 (4), 385–396.

Gulyas, B., Roland, P.E., 1994. Processing and analysis of form, colour and binocular disparity in the human brain: functional anatomy by positron emission tomography. Eur. J. Neurosci. 6 (12), 1811–1828.

Hadjikhani, N., Liu, A.K., Dale, A.M., Cavanagh, P., Tootell, R.B., 1998. Retinotopy and color sensitivity in human visual cortical area V8. Nat. Neurosci. 1 (3), 235–241.

Howard, R.J., Ffytche, D.H., Barnes, J., McKeefry, D., Ha, Y., Woodruff, P.W., Bullmore, E.T., Simmons, A., Williams, S.C., David, A.S., Brammer, M., 1998. The functional anatomy of imagining and perceiving colour. NeuroReport 9 (6), 1019–1023.

Ishihara, S., 1971. Tests for Colour-Blindness. Kanehara Shuppan, Tokyo.

Kastner, S., Crook, J.M., Pei, X., Creutzfeldt, O.D., 1992. Neurophysiological correlates of colour induction on white surfaces. Eur. J. Neurosci. 4, 1079–1086.

Komatsu, H., Ideura, Y., Kaji, S., Yamane, S., 1992. Color selectivity of neurons in the inferior temporal cortex of the awake macaque monkey. J. Neurosci. 12, 408–424.

Kosslyn, S.M., Thompson, W.L., Costantini-Ferrando, M.F., Alpert, N.M., Spiegel, D., 2000. Hypnotic visual illusion alters color processing in the brain. Am. J. Psychiatry 157 (8), 1279–1284.

McCollough, C., 1965. Color adaptation of edge-detectors in the human visual system. Science 149, 1115–1116.

McKeefry, D.J., Zeki, S., 1997. The position and topography of the human colour centre as revealed by functional magnetic resonance imaging. Brain 120, 2229–2242.

Meadows, J.C., 1974. Disturbed perception of colours associated with localized cerebral lesions. Brain 97 (4), 615–632.

Nunn, J.A., Gregory, L.J., Brammer, M., Williams, S.C., Parslow, D.M., Morgan, M.J., Morris, R.G., Bullmore, E.T., Baron-Cohen, S., Gray, J.A., 2002. Functional magnetic resonance imaging of synesthesia: activation of V4/V8 by spoken words. Nat. Neurosci. 5 (4), 371–375.

Riggs, L.A., White, K.D., Eimas, P.D., 1974. Establishment and decay of orientation-contingent after effects of color. Percept. Psychophys. 16 (3), 535–542.

Sakai, K., Watanabe, E., Onodera, Y., Uchida, I., Kato, H., Yamamoto, E., Koizumi, H., Miyashita, Y., 1995. Functional mapping of the human colour centre with echo-planar magnetic resonance imaging. Proc. R. Soc. Lond., B Biol. Sci. 261 (1360), 89–98.

Spitzer, H., Desimone, R., Moran, J., 1988. Increased attention enhances both behavioral and neuronal performance. Science 240 (4850), 338–340.

Talairach, J., Tournoux, P., 1988. Co-planar Stereotaxic Atlas of the Human Brain. Thieme, New York.

Wachtler, T., Sejnowski, T.J., Albright, T.D., 2003. Representation of color stimuli in awake macaque primary visual cortex. Neuron 37, 681–691.

Wade, A.R., Brewer, A.A., Rieger, J.W., Wandell, B.A., 2002. Functional measurements of human ventral occipital cortex: retinotopy and colour. Philos. Trans. R. Soc. Lond., B Biol. Sci. 357 (1424), 963–973.

Worsley, K.J., Friston, K.J., 1995. Analysis of fMRI time-series revisited—again. NeuroImage 2 (3), 173–181.

Zeki, S., 1983. Colour coding in the cerebral cortex: the responses of wavelength-selective and colour-coded cells in monkey visual cortex to changes in wavelength composition. Neuroscience 9, 767–781.

Zeki, S., 1990. A century of cerebral achromatopsia. Brain 113, 1721–1777.