Reciprocal Connectivity of Identified Color-Processing Modules in the Monkey Inferior Temporal Cortex

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The inferior temporal (IT) cortex is the last unimodal visual area in the ventral visual pathway and is essential for color discrimination. Recent imaging and electrophysiological studies have revealed the presence of several distinct patches of color-selective cells in the anterior IT cortex (AIT) and posterior IT cortex (PIT). To understand the neural machinery for color processing in the IT cortex, in the present study, we combined anatomical tracing methods with electrophysiological unit recordings to investigate the anatomical connections of identified clusters of color-selective cells in monkey IT cortex. We found that a color cluster in AIT received projections from a color cluster in PIT as well as from discrete clusters of cells in other occipitotemporal areas, in the superior temporal sulcus, and in prefrontal and parietal cortices. The distribution of the labeled cells in PIT closely corresponded with that of the physiologically identified color-selective cells in this region. Furthermore, retrograde tracer injections in the posterior color cluster resulted in labeled cells in the anterior cluster. Thus, temporal lobe color-processing modules form a reciprocally interconnected loop within a distributed network.

Keywords: color-selective cells, functional module, network, tracer

Introduction

The inferior temporal (IT) cortex is the last unimodal visual area in the ventral visual pathway (Ungerleider and Mishkin 1982). Although lesion studies have shown that the IT cortex plays a key role in color discrimination (Horel 1994; Heywood et al. 1995; Buckley et al. 1997; Huxlin et al. 2000; Cowey et al. 2001), the underlying machinery for the processing of color signals in the IT cortex is not understood. Previous single-unit studies in the anterior IT cortex (AIT) revealed that sharply color-selective neurons are clustered in a region posterior and dorsal to the anterior middle temporal sulcus (AMTS) (Komatsu et al. 1992; Yasuda et al. 2004; Koida and Komatsu 2007), referred as AIT color area (AITC) in this paper. Neuronal properties of the color-selective cells in this region were examined in detail. It has been shown that these cells can change their activity depending on the tasks monkeys are performing (Koida and Komatsu 2007) and that the activity of these cells and the behavior of the animals are correlated (Matsumura et al. 2008).

Recently, imaging studies have revealed that color-selective activities are not uniformly distributed across the IT cortex; instead, several patches of color-selective activation were observed in the AIT and posterior IT cortex (PIT) (Tootell et al. 2004; Conway and Tsao 2006; Conway et al. 2007; Harada et al. 2009). In line with these reports, we have found that sharply color-tuned cells are densely localized around the posterior middle temporal sulcus (PMTS), in what we have named the PIT color area (PITC) (Yasuda et al. 2010). These neurons are organized in a crude retinotopic map, and we proposed that the PITC is a color-processing region that includes a map of both the fovea and the periphery of the visual hemifield (Yasuda et al. 2010). Because the cells in PITC have similar color selectivity to the cells in AITC, the cells in PITC are likely candidates for an important source of color signals to AITC. To fully understand the complex machinery for color processing in the IT cortex, it is essential to elucidate the anatomical connectivity of these color-selective regions.

Several previous studies have suggested that the IT cortex consists of anatomically connected functional modules. Saleem et al. (1993) injected anterograde tracers in the PIT cortex and observed a patchy columnar terminal distribution that seemed to correspond to functional columns in the AIT cortex (Fujita et al. 1992). Based on this observation, these authors proposed that the projection from the PIT to AIT cortex is feature specific. In support of this hypothesis, Moeller et al. (2008) showed that several face-selective patches in the IT cortex form a mutually connected network.

In this report, we carried out several tracer injection experiments combined with electrophysiological identification of the color-selective regions. The basic question is from where does the AITC receive input and, more specifically, whether the PITC directly projects to the AITC. Our results indicate that AITC is in fact reciprocally connected with the PITC. The AITC received additional inputs from various other areas of the posterior occipitotemporal cortex, areas in the superior temporal sulcus (STS), and prefrontal and parietal cortices. Projections were found, however, to originate from subregions distinct from those that project to the neighboring AIT region, which is functionally distinct from the AITC. These results provide strong evidence that the color-selective regions in AIT and PIT are anatomically connected with each other and form a functional network for color vision.

Materials and Methods

All procedures for animal care and experimentation were in accordance with the National Institutes of Health Guide for the Care and Use
of Laboratory Animals and were approved by the Institutional Animal Care and Use Committee of National Institute of Natural Sciences. Four adult Japanese monkeys (Macaca fuscata) weighing 4.65–7.70 kg were used in this study. Tracer placement was guided by sulcal patterns and/or by electrophysiological data. Neuronal activity was recorded from 3 monkeys (monkeys LS, KS, and PI) for which a head fixation post, recording chambers, and a scleral search coil were implanted. Surgeries were conducted using aseptic techniques under sodium pentobarbital general anesthesia.

Detailed surgical procedures have been previously described (Yasuda et al. 2010). Briefly, delrin chambers (16 × 16 or 21 × 21 mm²) were fixed to the skull at the positions corresponding to either the PIT cortex around the PMTs or the AIT cortex around the posterior end of the AMTS. In monkeys LS and PI, the chamber position was determined based on magnetic resonance (MR) images taken before the surgery. In monkey KS, an MR image was not available, and the chamber position was determined based on stereotaxic coordinates. After surgery, animals were allowed to recover for at least 1 week before the electrophysiological recordings. During this period, an antibiotic (Cefazolin sodium) was given every 12 h.

In monkeys LS, KS, and PI, detailed recordings were carried out over 5–10 months. Subsequently, we injected retrograde tracers (cholera toxin subunit B conjugated with Alexa Fluor 488 [CTB-Alexa488; Invitrogen-Molecular Probes] or CTB conjugated with Alexa Fluor 555 [CTB-Alexa555; Invitrogen]) or an anterograde tracer (biotinylated dextran amine [BDA; Invitrogen]) in the electrophysiologically mapped regions. In monkey HP, we did not perform electrophysiological recordings, and CTB-Alexa488 was injected using sulcal landmarks. The recording sites, and types and sites of the tracer injections are summarized in Table 1. The results of the recordings from PIT in monkey LW are partially reported in our previous paper (Yasuda et al. 2010).

### Electrophysiology

Recordings were made through implanted chambers located at the AIT and/or PIT cortex. A varnish-coated tungsten microelectrode (Frederick Haer) was penetrated through a stainless guide tube, and single- or multiple-unit activities were recorded. In order to map the neurons systematically, we used a grid with an array of holes at 1-mm interval. Occasionally, we penetrated electrodes in the middle of the arrays by using another grid that contains an array of 0.5 mm shifted holes. Neurons were tested for their color and shape selectivity and mapped on the grid coordinates. The electrodes were advanced by a hydraulic microdrive (Narishige), and neurons were sampled at a 100- to 300-μm interval in each penetration. Neuronal signals were amplified, sampled at 25 kHz, and stored on a computer for offline analysis. The amplified signals were also fed into a speaker, and the visual response was monitored online by ear.

In the recordings from the AIT cortex, electrodes were penetrated vertically from the chamber at the top of the skull. After every experimental session, the trajectory of the electrodes was checked by taking an X-ray (Toshiba TR-80A-ES-L; 70 kV, 20 mA, 0.4 s) while the electrode was still in the brain, to confirm location and that the recording electrode was not bent before reaching the targeted cortex in the IT gyrus. In the recordings from PIT cortex, the targeted cortical site was immediately subjacent to the dura exposed in the recording chamber, and the X-ray was therefore considered not necessary. In monkey LW, we made 3 electrolytic lesion markings (−10 μA; 60 s) at the final stage of the recording session in PIT. These markings were later used for the identification of the recording sites (see Alignment of Physiological Map and Unfolded Density Map).

### Visual Stimulus and Task Sequence

We used the same set of color and shape stimuli as used in a previous experiment (Yasuda et al. 2010). Stimuli were presented on a neutral gray background. The stimulus set consisted of 15 or 14 colors and 11 simple geometrical shapes (Fig. 1). The luminance of the stimulus was either 5 or 20 cd/m², and the background was 10 cd/m². The 20 cd/m² stimulus set consisted of 14 colors because the maximum luminance of the most blue stimulus (#15) was 11.8 cd/m². All stimuli were presented on a CRT monitor (Sony GDM-F500R, 800 × 600 pixels, 40° horizontally × 30° vertically; 144 frames/s).

During the experiment, monkeys were seated in a primate chair facing the CRT monitor at a distance of 56 cm. The monkeys were trained to fixate on a small white dot presented at the center of the monitor. Trials were started when the monkeys began to fixate on the fixation spot. After 500 ms of fixation period, a visual stimulus was presented on the monitor. The stimulus period lasted for 500 ms, and a blank period (260 ms) followed. When the visual stimulus was presented at the foveal center, the fixation spot was turned off for a period extending from 350 ms before visual stimulus onset until 260 ms after stimulus offset. When the stimulus was presented elsewhere in the visual field, the fixation spot was turned on during the entire trial. During the trials, the monkey’s gaze was monitored by scleral search coil or by an infrared eye tracking system (ISCAN). If the monkeys successfully maintained fixation within a 3 × 3° window throughout the trial, a drop of juice was given as a reward. If their gaze deviated from the window during a trial, the trial was aborted. The behavioral

### Table 1

<table>
<thead>
<tr>
<th>Monkey</th>
<th>Recording site</th>
<th>Injection site</th>
<th>Tracer</th>
<th>Amount (μL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HP</td>
<td>No recording</td>
<td>AIT</td>
<td>CTB-Alexa488</td>
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<td>LW</td>
<td>AIT and PIT</td>
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<td>CTB-Alexa488</td>
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<tr>
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<td>PIT</td>
<td>PIT</td>
<td>CTB-Alexa488</td>
<td>0.5</td>
</tr>
<tr>
<td>PI</td>
<td>PIT</td>
<td>BDA</td>
<td></td>
<td>1.2</td>
</tr>
</tbody>
</table>

Figure 1. The standard stimulus set used in the electrophysiological experiment. (A) The color set consists of 15 colors evenly distributed over the CIE 1931 xy chromaticity diagram. The 15 numbered points indicate the coordinate of color stimuli on the chromaticity diagram. (B) The shape set consists of 11 simple geometric shapes including square, diamond, circle, star, cross, oblique cross, triangle, and 4 oriented bars (differing by 45°). The luminance of the stimulus is either 5 or 20 cd/m², and each of the visual stimulus is composed of a combination of color and shape from these standard sets.
Data Analyses: Electrophysiology

The detailed quantitative offline analysis has been described previously (Yasuda et al. 2010). Single neurons were isolated using a template matching algorithm and average firing rate was calculated. We computed the average firing rate by subtracting baseline activity (0–300 ms before stimulus onset) from the average firing rate during the stimulus presentation period (50–550 ms after stimulus onset). Neurons that showed significant activity (Student’s t test, $p < 0.05$) in the stimulus presentation period were included in the analysis as visually responsive neurons. Even if the neuronal response was statistically significant, the neurons that had maximum firing rate of less than 10 spikes/s were considered visually unresponsive and were omitted from the analysis.

For visually responsive neurons, we evaluated stimulus selectivity by using one-way analysis of variance. We also evaluated the sharpness of the stimulus selectivity by computing a sparseness index ($\text{ SPI } = 1 - \sum r^n / n$, where $r$ is the firing rate to the stimulus and $n$ is the stimulus set size). If $r$ was negative, it was replaced by zero. If the SPI was greater than or equal to 0.3, the neuron was considered to be sharply tuned to the stimulus. In recordings from the PIT cortex, we also determined the receptive field (RF) of the cells. We presented the optimal stimuli at various locations on the display and then determined online the vertical and horizontal extents where responses could be evoked. The criteria for the sharpness of the selectivity, and the RF determination were identical to those in the previous report (Yasuda et al. 2010).

Color-selective regions are concentrated in the subregion of AIT near the AMTS (AITC) and the subregion of PIT near the PMTS (HTC). To accurately inject tracers in the color-selective regions, in this report, we defined “color clusters” within AITC and/or PITC as the tracks where most of the neurons were sharply color tuned, but not selective for, or only broadly tuned for shape. To identify the color clusters, we calculated the average of the SPI for color and shape for each penetration site. The penetration site was scored as a color cluster if the averaged SPI for color was equal to or greater than 0.3, but that for shape was less than 0.3.

Tracer Injections

Using sulcal landmarks (in monkey HP) and/or the results of the electrophysiological recordings (in monkeys LW, KS, and PI), injection sites were determined. We used CTB-Alexa488 or CTB-Alexa555 as retrograde tracers and BDA as an anterograde tracer. The retrograde tracers, CTB-Alexa488 and CTB-Alexa555, were diluted at 1% concentration in 0.1 M phosphate buffered saline (PBS). BDA of molecular weight 3000 and 10 000 were mixed in a 1:1 proportion and diluted to 10% in 12.5 mM PBS.

In monkey HP, we injected 1.0 $\mu$L of CTB-Alexa488 in the posterior dorsal end of the AMTS. The injection site was determined only with reference to the AMTS because we did not record neuronal activity from this monkey. We removed a piece of skull covering the lateral surface of the AIT cortex to directly visualize the AMTS and injected retrograde tracer under direct visualization. In monkey LW, 0.5 $\mu$L of CTB-Alexa488 was injected in an electrophysiologically identified color cluster of the AIT cortex. We removed a piece of skull covering the lateral surface of the AIT cortex, exposed the dura over the posterior end of the AMTS, and made a small slit through the dura. An electrode was penetrated from the recording chamber attached on the top of the skull until the electrode tip could be detected at the surface of the IT gyrus. After identifying the tip of the electrode through the slit in the dura, we photographed the position of the tip and withdrew the electrode. Using the blood vessel pattern as a guide, the injection needle was inserted into the targeted position perpendicularly from the cortical surface. In addition to CTB-Alexa488, we also injected 0.5 $\mu$L of CTB-Alexa555 at a site 2.5 mm dorsal to the first injection site. This site was −2 mm lateral to the CTB-Alexa488 injection site in the grid coordinate and was outside of the physiologically identified color cluster.

In monkey KS, 0.5 $\mu$L of CTB-Alexa488 was injected in the electrophysiologically identified color cluster of the PIT cortex. As was the case with monkey KS, the injection was targeted to cortex beneath the dura in the recording chamber. We made a slit in the dura and marked the cortical site corresponding to the targeted grid coordinate by black ink. The tracer was injected guided by this marking.

In all the cases except monkey KS, the tracers were pressure injected through a 50-μm-diameter glass micropipette attached to a 10-μL Hamilton syringe. In the case of monkey KS, the pressure injection was done through a 10-μL Hamilton syringe containing a recording electrode inside the needle (MRM-502; Crist Instrument). This allowed us to monitor neuronal activity and inject the tracer simultaneously. In the case of injections in the AIT cortex, after the injection, cortex was covered by an artificial dura (Preclude Dura Substitute; W.L. Gore & Associates, Inc.) and the skin wound was sutured closed. After the injections, the monkeys were recovered and returned to the cage.

Fixation and Tissue Preparation

After survival periods of 21 (monkey HP), 14 (monkey LW), 13 (monkey KS), and 21 (monkey PI) days, the monkeys were anesthetized with ketamine hydrochloride (intramuscularly 1.0 cc) and sodium pentobarbital (overdose; 0.7 cc/kg intraperitoneally and 5.0 cc intracardially) and perfused transcardially, in sequence, with 1 L of saline (3 min), 4 L of 4% paraformaldehyde in 0.1 M phosphate buffer (PB; pH 7.4; 30 min), and 0.5 L each, of chilled 10%, 20%, and 30% sucrose in 1.0 M PB. For monkeys where the chamber was implanted over PIT cortex, several markings were made on the PIT cortex by ink. Before removing the brain from the skull, the dura inside of the recording chamber over the PIT cortex was removed, and the markings were made by a guide tube through a grid. The ink-marked cortex was photographed, and these photos were used for identification of the injection site and other penetration sites. In monkey LW, these markings were also used for the alignment of the electrophysiological data and anatomical data (see Alignment and Physiological Map and Unfolded Density Map). After photographing, the brains were removed, trimmed, and placed in 30% sucrose in PB solution for a few days until they sank (at 4 °C).

After the brains sank, the brain blocks were coronally sectioned on a freezing microtome at 50 μm thickness. In monkeys HP, LW, and KS, the sections were divided into 5 repeating series. In monkey HP, the first series was further divided into 2 repeating series, where half were stained for Nissl substance by thionin, and the remaining half was reacted for SMI-32. The second and third series were reacted for CTB-Alexa488 by immunoperoxidase, and the third series was further counterstained with neutral red. The fourth series was used to enhance the CTB-Alexa488 signal by immunofluorescence (anti-alex488), and the fifth was reserved for other purposes. In monkey LW, the first series was double reacted for Nissl substance and CTB-Alexa488 by immunoperoxidase. The second series was used for the signal enhancement for CTB-Alexa488 by immunofluorescence. The third, fourth, and fifth series were reacted for Nissl substance by thionin, for CTB-Alexa488 by immunoperoxidase, and for parvalbumin (PV) by immunoperoxidase, respectively. For CTB-Alexa555, no enhancement was used. In monkey KS, the first and second series were reacted for CTB-Alexa488 by immunoperoxidase and the second series were further stained for Nissl substance by thionin. The remaining sections were kept for other purpose or discarded. In monkey PI, all the sections were reacted for BDA by 3.5 diamobenzidine tetrahydrochloride (DAB) and peroxidase.

Immunoperoxidase Reaction for CTB-Alexa488

Sections were immunoblocked in 0.1 M PBS, pH 7.4, containing 0.5% Triton X-100 and 5% normal goat serum (PBS-TG) for 1 h at room temperature.
temperature and subsequently incubated with 1:1000 rabbit anti-Alexa488 antibody (Invitrogen) in PBS-TG for 2 days at 4 °C. After washing with 0.1 M PBS, sections were incubated for 1.5 h at room temperature in biotinylated antirabbit polyclonal goat antibody (1:200; Vector Laboratories). Immunoreactivity was visualized by avidin biotinylated peroxidase complex (ABC) incubation (one drop of reagents per 7 mL of 0.1 M PB, ABC Elite kits; Vector Laboratories) followed by diaminobenzidine histochemistry with 0.03% nickel ammonium sulfate.

Enhancement of CTB-Alexa488 Signal by Immunofluorescence Sections were immunoblockered in PBS-TG for 1 h at room temperature and subsequently incubated with 1:1000 rabbit anti-Alexa488 antibody (Invitrogen) in PBS-TG for 2 days at 4 °C. After washing with 0.1 M PBS, sections were incubated for 1.5 h at room temperature in Alexa488-conjugated antirabbit polyclonal goat antibody (1:200; Invitrogen-Molecular Probes).

Histochemistry for BDA Sections were incubated in 0.1 M PBS, pH 7.4, containing 0.5% Triton X-100 for 1 h at room temperature and subsequently incubated for 20–24 h in ABC solution (Vector Laboratories) at room temperature. Subsequently, the reactivity was visualized by diaminobenzidine histochemistry with 0.03% nickel ammonium sulfate.

Immunoperoxidase Reaction for SMI-32 and PV Sections were incubated for 1 h with PBS-TG at room temperature and then for 40–48 h at 4 °C with PBS-TG containing anti-SMI-32 monoclonal mouse antibody (1:800; Sternberger Monoclonals) or anti-PV monoclonal mouse antibody (1:50 000; Swant). After rinsing, the sections were placed in PBS-TG containing biotinylated anti-mouse immunoglobulin G polyclonal goat antibody (1:200; Vector Laboratories) for 1.5 h at room temperature. Immunoreactivity was visualized by ABC incubation (Vector Laboratories) followed by diaminobenzidine histochemistry with 0.03% nickel ammonium sulfate.

All sections were mounted on gelatin-coated glass slides, air-dried, dehydrated in graded ethanol solutions, immersed in xylene, and coverslipped in DPX (Fluka).

Data Analysis: Anatomy Tissue sections were scanned with a light microscope using lower magnification (×100 or ×200), and the distribution of the labeled cells or terminals was noted. For the confirmation of terminal specializations, we used higher magnification (×400 or ×1000). Fluorescent signal was analyzed by using standard sets of filters: fluorescein (for CTB-Alexa488) or rhodamine (for CTB-Alexa555). Injection size was defined according to previous studies (Ippino et al. 2003; Borra et al. 2010). The core of the injection site, which is the effective tracer uptake area, for CTB-Alexa488 and CTB-Alexa555 was considered to include the intensely fluorescent area around the needle track. The contours of the cortical surface and the border between the white matter and gray matter were traced; and the position of the labeled cells and axon terminals were plotted using Neurolucida system (MicroBrightField). The plotted series of coronal sections is shown in Supplementary Figures 3 (for monkey HP) and 4 (for monkey LW). For convenience, we mainly used the brightfield DAB sections for the examination of the labeled cell distribution of CTB-Alexa488. In monkey LW, the labeled cell distribution of CTB-Alexa488 was examined in fluorescein, and a pair of adjacent sections was used for the comparison of the labeled cell distributions of CTB-Alexa488 and CTB-Alexa555.

Areas V1, V2, V4, and TEO were identified by reference to sulcal landmarks in ABC, in comparison with published maps, and by architectonic analysis of selected histological sections stained for cell bodies, PV, or SMI-32 (Saleem and Logothetis 2007; Ungerleider et al. 2008; Borra et al. 2010). Subdivisions within the IT cortex followed the descriptions of Yukie et al. (1990), Saleem and Tanaka (1990), Saleem et al. (2007), and Saleem and Logothetis (2007), with reference to that of Suzuki and Amaral (2003a, 2003b). Foci of labeled neurons were described within area TF, TH, TG, and smaller subdivisions of TE within or near the STS (i.e., TEm, TEa, and TPO). These were designated with reference to Saleem and Logothetis (2007) Seltzer and Pandya (1989), and Casick et al. (1995).

To visualize the distribution and density of labeled neurons throughout the occipitotemporal region, we unfolded the traced cortical surface (Van Essen and Maunsell 1980), and charted raw data on the unfolded cortex on which boundaries of visual areas are delineated as described above (Supplementary Fig. 1). The 2-dimensional reconstructions of the cortical surface were obtained by unfolding contours of layer 4 of sections 500 μm apart in a way so as to keep the neighborhood relationships between and within sections. The contour lines of layer 4 were divided into 500-μm intervals, and the cortex was divided into 500 μm columns perpendicular to the layer 4. We manually counted the labeled cells in each of the columns. The columns that contained at least one labeled cell were sorted with regard to the number of cells in ascending order, and the number of cells in the 95th percentile columns was identified (the 95th percentile count). The percentage relative to the 95th percentile count was computed for each column, and this value was visualized by a color code on the sulcus-unfolded map.

In the case of the BDA injection, fields with terminal boutons were traced on the slices at 500-μm intervals by the Neurolucida system. The cortex was divided into columns of 500 μm widths, and the density of boutons in each column was subjectively categorized into 3 levels (dense, intermediate, and sparse). The values were projected on the layer 4 and superimposed on the unfolded cortex obtained by the aforementioned technique. As corroboration of this method, we counted BDA-labeled boutons sampled through 10 regions within IT cortex, and plotted results as number of boutons per 100 × 100 μm² in relation to the subjectively assigned density levels (Supplementary Fig. 5).

In monkey LW, we made a more detailed density map around the PMTs in addition to the map for the entire occipitotemporal region, applying similar techniques to what we used in the preparation of the sulcus-unfolded map of the occipitotemporal cortex. The resolution of this map was 250 × 250 μm², and the distribution of the labeled cells in the PIT cortex was closely compared with that of color-selective cells revealed by electrophysiological mapping.

Alignment of Physiological Map and Unfolded Density Map In the case of monkey LW, retrograde tracer was injected at the AIIT site where a color cluster was electrophysiologically identified. At the same time, we also recorded and identified the location of color clusters in the PIT cortex. To evaluate the relationship between the color clusters and the distribution of the labeled cells in the PIT cortex, we prepared a sulcus-unfolded density map of the labeled cells, as well as the electrophysiological map represented on the grid coordinate, and aligned these by using fiducial marks. In the final stage of recording sessions from the PIT cortex, we made markings by electrolytic lesion in 3 penetration sites, and these lesions were identified on the processed brain sections (EL1-3 in Supplementary Fig. 2). In addition, after the perfusion, we put 3 additional markings by ink on the cortical surface at the sites corresponding to the 3 electrode penetration sites (IM1-3 in Supplementary Fig. 2). Using these markings, we identified the rotation angle of the recording chamber with respect to the gross brain and the slice plane of the fixed brain. The lesion and ink fiducial points further assisted in shrinkage correction and mapping the recording sites to the tissue sections. Each of the assigned recording sites was projected onto layer 4 of the corresponding tissues and superimposed on the sulcus-unfolded density map of the labeled cells.

Photographic Presentation The photomicrographs shown in the present study were obtained by capturing images directly from the sections using a digital camera (DP 50; Olympus) attached to the microscope (BX51; Olympus). In monkey HP, we also photographed the injection site before slicing the brain block. The fluorescent image was obtained by a digital camera (Penguin 600CI; Pixera) attached to a fluorescence binocular stereomicroscope (MZ FLUORIII; Leica). Individual images are imported into Adobe Photoshop (Adobe Systems Incorporated). The images were then processed, assembled into digital montages, and reduced to the final size.
Results

Case 1: Monkey HP

Single-unit studies of monkey IT cortex have repeatedly reported a region where strongly color-selective cells are accumulated just posterior and dorsal to the AMTS (A1T1C) (Komatsu et al. 1992; Yasuda et al. 2004; Koida and Komatsu 2007; Matsumora et al. 2008). Recent imaging studies also show color-related signals around the posterior tip of the AMTS (Tootell et al. 2004; Harada et al. 2009). Although the position and the shape of the AMTS were slightly variable across our 4 monkeys, we found the sulcus to be a helpful landmark for the A1T1C. We were particularly interested to know whether there is a direct connection between the region around the PMTS, where a cluster of color-selective cells was also reported (PIT1C), and the A1T1C. To explore this possibility, we injected 1.0 μL of retrograde tracer, CTB-Alexa488, at the posterior tip of the AMTS (Fig. 2A,B), where A1T1C is located. The core of the

Figure 2. Injection site and distribution of the labeled cells in monkey HP. (A) Lateral view of the brain of monkey HP. The black dot near the AMTS indicates the injection site, and the arrow indicates a region at the ventral lip of the PMTS where a dense cluster of labeled cells was observed. (B) Fluorescence photograph of the gross brain focusing on the IT cortex around the injection site. The core and halo of injection site of a retrograde tracer CTB-Alexa488 is visible just posterior and dorsal to the AMTS. The intense injection core has saturated the optics and appears almost white. The labeled cells at the ventral tip of the PMTS are weakly visible from the surface (arrow). Scale bar = 5 mm. (C) Micrograph of the labeled cells corresponding to the cluster of cells indicated by arrow in (A) and (B). Scale bar = 250 μm. (D) Distribution of the labeled cells after a tracer injection, now shown in a sulcus-unfolded representation of left occipitotemporal cortex. Solid lines indicate the ridge of main sulci, and broken lines indicate their fundi or floor. The number of labeled cells within 500 x 500 μm² regions is indicated as a percentage of the 95th percentile value of nonzero counts (95th percentile count: 224 cells per unit area) using the color scale shown on the right. The total number of cells counted in monkey HP is 90 644. The tracer injection site is indicated by a black circle. The cluster of labeled cells at the ventral tip of the PMTS shown in (A–C) is indicated by an arrow. Scale bar = 5 mm. AMTS, anterior middle temporal sulcus; LS, lunate sulcus; OTS, occipitotemporal sulcus; PMTS, posterior middle temporal sulcus; RS, rhinal sulcus; STS, superior temporal sulcus.
injection site was well confined to the tissue posterior and dorsal to AMTS, and the diameter of the effective tracer uptake area was 650 μm.

After this injection, labeled cells were distributed widely within the IT cortex (Supplementary Fig. 3), but their distribution was not homogeneous. We found a dense concentration of labeled cells at the ventral lip of the PMTS. Fluorescent signal from this region could be observed even on the surface of the gross brain, without further processing (Fig. 2B arrow), and corresponded to the dense cluster of labeled cells, as verified by histological processing (Fig. 2C). Dorsal to the PMTS, however, only a few labeled cells were observed. The distribution of the labeled cells was contained within, but not entirely coincident with the region where we previously reported a dense cluster of color-selective cells (PITC: Yasuda et al. 2010). In Yasuda et al., we reported sharply color-tuned cells, both dorsal and ventral to the PMTS. This result suggests direct projections from PITC to AITC, but with a difference in the projection pattern between the regions dorsal and ventral to the PMTS.

The entire distribution of the labeled cells in the occipito-temporal cortex, as determined by microscopic inspection of the coronal sections (at intervals of 500 μm; Supplementary Fig. 3) is shown in Figure 2D as a sulcus-unfolded density map. In addition to the region ventral to the PMTS, labeled cells were observed in areas TG, TEav, TEad, TEpv, and TEpd in the IT gyrus. In STS, the labeled cells were distributed in areas TPO, TEM/TEa, PGa, IPa, FST, PITd, and V4t. We also observed labeled cells in area 36 of the perirhinal cortex and areas TF, TH, and TFO of the parahippocampal cortex. Some labeled cells were also observed in area V4, especially in the ventral part (see also Table 2). We also found small foci of labeled cells in the prearcuate convexity and orbital surface of the prefrontal cortex. These results are highly consistent with previous tracer injection studies (Morel and Bullier 1990; Webster et al. 1991, 1994; Borra et al. 2010). Of these regions, the labeled cells were densest in the lower bank of the STS and at the ventral lip of the PMTS (Fig. 2D).

**Case 2: Monkey LW**

In the second monkey, we set out to corroborate the projection from the PITC to AITC, with the addition of direct physiological mapping of the color and shape selectivity of cells in both the AIT and the PIT cortex. We recorded 47 visually responsive neurons in the AIT cortex and examined color and shape selectivity of these cells using a set of color stimuli with
simple geometrical shapes (Fig. 1). The distribution of the color- and/or shape-selective cells was mapped on a grid coordinate within the recording chamber through which electrodes penetrated vertically to AIT cortex.

Of these neurons, we quantitatively evaluated the selectivity sharpness for 25 cells by calculating an SPI. A typical AIT neuron that was selective for color but was not selective for shape is shown in Figure 3. Consistent with previous studies (Komatsu et al. 1992; Yasuda et al. 2004; Koida and Komatsu 2007), the color-selective cells were clustered near the posterior end of the AMTS and slightly lateral to it (Fig. 4A,B). In particular, we found a spot about 3×3 mm² in extent where most cells were color selective but not shape selective (enclosed by a thick line in Fig. 4B). Within this spot, the average of the color SPI at each site was equal to or greater than 0.3 and that of shape was less than 0.3. By this criterion, we considered this spot as an AIT color cluster. The cells from the most posterior and medial penetration sites tended to be difficult to activate by our stimulus parameters (Fig. 4B), and we could not evaluate color and shape selectivity.

In the PIT cortex, we recorded 220 visually responsive neurons and quantitatively analyzed 127 neurons. We identified a region having sharply color-tuned neurons and a crude retinotopic organization (PITC) (Yasuda et al. 2010). The PITC extended both dorsal and ventral to the PMTS. In the dorsal region, neurons had RFs centered at the fovea. In the ventral region, the RF center shifted to the peripheral visual field. Neurons in the posterior part had RFs in the upper visual field, whereas those in the anterior part had RFs in the lower visual field (Fig. 5A). We computed the SPI of both color and shape for the PITC neurons. Sharply color-tuned but broadly shape-tuned cells were less tightly clustered than in the AIT cortex, but we could identify 2 distinct color clusters in the PITC by using the same criteria as we used in the AIT (Fig. 5B). One color cluster was located ventral to the PMTS including its ventral bank. The cells in this color cluster had large RFs including both fovea and periphery (Fig. 5A,B). The other color cluster was located more dorsally and slightly anterior to the inferior occipital sulcus (IOS). The cells in this color cluster had smaller RFs centered at the fovea (Fig. 5A,B).

After the electrophysiological mapping in both AIT and PIT had been completed, CTB-Alexa488 was injected at the center of the color cluster identified in AIT, as determined by the physiological map (Fig 4C). In addition, CTB-Alexa555 was injected 2.5 mm dorsally from the center of the color cluster. This site can be projected as ~2 mm lateral to the color cluster in the grid coordinate and corresponded to the region outside of the electrophysiologically identified color cluster. The injection site of CTB-Alexa555 was slightly larger (550 μm) than that of CTB-Alexa488 (375 μm), but there was no overlap in the injection cores or halo (Fig. 4C). After histological processing, labeled cells were observed along the IT cortex (Supplementary Fig. 4) including the region around PMTS. In this region, we found both CTB-Alexa488- and CTB-Alexa555-labeled cells, but these formed separate clusters with negligible overlap. This result indicates that the population of PIT cells is strongly segregated in the projection to the 2 injection sites in the AIT cortex (located 2.5 mm away from each other, center-to-center).

To examine the relationship between the distribution of the labeled cells and the color clusters in PITC, we superimposed the electrophysiologically map onto the sulcus-unfolded projection density map of the PMTS (Fig. 5C and Supplementary Fig. 2). By this procedure, we found multiple small (~1×1 mm²) clusters of CTB-Alexa488-labeled cells within the site corresponding to the PIT color clusters. In contrast, the CTB-Alexa555-labeled cells were rarely observed within the established boundaries of the PIT color clusters, although they were occasionally observed at the edge. This impression was confirmed when we computed the ratio of the labeled cells in the color clusters in relation to the total number of labeled cells in the posterior recording site: the ratio was much smaller for Alexa555 (351/2865 = 12.3%) than for Alexa488 (139/337 = 41.2%).

This result supports the conclusion that the AITC receives input from the PITC. Interestingly, the projection neurons mainly arose from the ventral color cluster but not from the dorsal color cluster in PITC. The finding that the projection from PIT to the AITC preferentially arises from the region ventral to the PMTS is consistent with the observation in monkey HP, and this supports the interpretation that the projection patterns differ between the regions dorsal and ventral to the PMTS.

The distribution of the labeled cells in the occipitotemporal cortex for monkey LW is shown in Figure 6B as a sulcus-unfolded density map, and representative sections are shown in Figure 6A. The location of the labeled cells was highly consistent with the results in monkey HP and with previous anatomical studies for both tracers (Morel and Bullier 1990; Webster et al. 1991, 1994; Borra et al. 2010). Other than TEO, we found labeled cells in areas TG, TEd, TEar, TEdp, and TEpv in the IT cortex for both tracers (see also Table 3). Labeled cells were also observed in area 36 of the perirhinal cortex and areas TF, TH, and TFO in the parahippocampal cortex. In the STS, labeled cells were observed in areas IPa, TEm, TEa, and FST, but no labeled cells were observed in TEd and V4t. Posterior to the IT cortex, we found labeled cells in area V4v for both tracers. The labeled cells were not uniformly distributed within these areas but tended to be in distinct clusters. Small foci of labeled cells were detected in the ventrolateral and orbital surface of prefrontal cortex and in the lateral bank of the intraparietal sulcus. Both CTB-Alexa488- and CTB-Alexa555-labeled cells were observed in the ventrolateral prefrontal cortex, and the distribution was largely overlapped. Only CTB-Alexa488-labeled cells were observed in the orbito-prefrontal cortex and parietal cortex. Because the size of the injection site was larger for CTB-Alexa555 than for CTB-Alexa488, the total number of the CTB-Alexa555-labeled cells was much larger than that of the CTB-Alexa488-labeled cells. However, in the STS, cells labeled by CTB-Alexa488 were dominant, despite the smaller size of the injection. The injection site of CTB-Alexa555

<p>| Table 2 |
| Quantitative analysis of retrogradely labeled neurons in occipitotemporal cortex in monkey HP |</p>
<table>
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<tr>
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<th>TEav</th>
<th>TEpd</th>
<th>TEpv</th>
<th>TEO</th>
<th>TG</th>
<th>A36/35</th>
<th>TF/TH</th>
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<tr>
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<td>IPa</td>
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<td>FST</td>
<td>PITd</td>
<td>V4t</td>
<td>V4</td>
<td>V2/V3</td>
<td>Total</td>
</tr>
<tr>
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<tr>
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<td>2876</td>
<td>2355</td>
<td>19</td>
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<td>5</td>
<td>90 644</td>
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<tr>
<td>%</td>
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<td>0.91</td>
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<td>3.17</td>
<td>2.60</td>
<td>0.02</td>
<td>0.56</td>
<td>&lt;0.01</td>
<td>100</td>
</tr>
</tbody>
</table>

Note: Total numbers and percentages are given by area. % = percentage of retrogradely labeled cells in one area/total labeled cells in the occipitotemporal cortex shown in the sulcus-unfolded density map.
was 2.5 mm dorsal to the injection site of CTB-Alexa488, and this dorsoventral relationship was generally preserved in the disposition of labeled cells posterior to the injection sites. The overlap of the distributions in the IT cortex was small in general but slightly larger in the region anterior to the injection sites.

**Case 3: Monkey KS**

Although the reciprocal connectivity between AIT and PIT has been documented (Morel and Bullier 1990; Webster et al. 1991; Distler et al. 1993; Suzuki et al. 2000), specific details are less well established about the topography of the projections between small local regions. Because the projection from AIT to PIT is divergent (Suzuki et al. 2000), a retrograde tracer injection in one focal site of PIT might be expected to label a divergent population of cells in AIT, some of which would have dissimilar functional properties compared to the cells in the posterior injection site. Alternatively, the projections may still specifically connect functionally similar groups of cells. In some support of this hypothesis, Moeller et al. (2008) have recently reported reciprocal connections specifically between face patches in monkey IT cortex.

To examine the feedback connectivity of the color-selective regions in the AIT and PIT cortex, we injected a retrograde tracer (CTB-Alexa 488) after electrophysiologically identifying a color cluster in PTC (Fig. 7). We recorded 105 visually responsive cells and quantitatively analyzed 11 neurons. In this monkey, color-selective neurons were restricted to a smaller region in PIT than in monkey LW. Nevertheless, consistent with the results of monkey LW and our previous report (Yasuda et al. 2010), we found that sharply color-selective cells were concentrated around the PMTS (enclosed by thick line in Fig. 7B). The color-selective cells in this region had large RFs including the fovea and the region showed a crude retinotopy (Fig. 7A), which is similar to the organization described for LW and to that reported previously in the PIT cortex (Boussaoud et al. 1991; Yasuda et al. 2010). Because color-selective neurons were concentrated in a relatively small region, we could not differentiate ventral and dorsal color clusters as in monkey LW. Judging from the location relative to the PMTS, however, we considered the color-selective region as comparable to the ventral color cluster in monkey LW.

We injected 0.5 μL of retrograde tracer CTB-Alexa488 in this physiologically identified color cluster (Fig. 7C). The size of the injection site in monkey KS was 500 μm in diameter, much smaller than the extent of the color cluster in this hemisphere.

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**Figure 4.** Electrophysiological mapping at the posterior edge of the AMTS and the tracer injection sites in monkey LW. (A) Ventral view of AIT cortex. Circles indicate penetration of the recording electrodes, as projected onto the cortical surface. (B) Response properties of neurons at each of the penetration sites, as shown in (A). For the penetrations where quantitative analysis of color and shape selectivity was carried out, SPs were computed. The SPs for color and shape are separately averaged over each of the penetration sites and are shown as pairs of numbers on the corresponding grid coordinate of the map. The numbers at the top are SPs for color and those at the bottom are SPs for shape. The penetration sites are classified based on these averaged SPs. If the averaged SPs for both color and shape are greater than or equal to 0.3, the corresponding coordinates are shaded. The meshed coordinates indicate penetration sites where the SP for color was greater than or equal to 0.3 and that for shape less than 0.3. The dotted coordinates indicate penetration sites where the SP for color was less than 0.3 and that for shape greater than or equal to 0.3. For several recording sites where neurons were examined only qualitatively, the selectivity of each individual neuron is shown by symbols (filled star, both color and shape selective; filled circle, color selective but not shape selective; open star, shape selective but not color selective; and open circle, neither color nor shape selective). Sites where no significant response was observed are shown by x. The color cluster, identified as the locus where cells had sharp color selectivity but broad shape selectivity, is enclosed by a thick contour. Tracer injection sites are indicated by arrows. (C) The injection sites of CTB-Alexa488 (at right) and CTB-Alexa555 (at left). The amounts of the tracers are 0.5 μL. Scale bar = 1 mm.
Figure 8A shows the distribution of the retrogradely labeled cells. In the IT cortex anterior to the injection site, the labeled cells formed 2 clusters. One was located posterior and dorsal to the AMTS and the other cluster was located at the lateral lip of the occipitotemporal sulcus (OTS). Although we had not electrophysiologically identified the AITC in this monkey, the location of the cluster of labeled cells near the AMTS corresponds to the site where color-selective cells have been observed repeatedly (Komatsu et al. 1992; Yasuda et al. 2004; Koida and Komatsu 2007; Matsumora et al. 2008) and where we demonstrated the AITC in monkey LW. This supports the idea that AITC and PITC are reciprocally connected.

Figure 8B shows the distribution of the retrogradely labeled cells. In the IT cortex anterior to the injection site, the labeled cells formed 2 clusters. One was located posterior and dorsal to the AMTS and the other cluster was located at the lateral lip of the occipitotemporal sulcus (OTS). Although we had not electrophysiologically identified the AITC in this monkey, the location of the cluster of labeled cells near the AMTS corresponds to the site where color-selective cells have been observed repeatedly (Komatsu et al. 1992; Yasuda et al. 2004; Koida and Komatsu 2007; Matsumora et al. 2008) and where we demonstrated the AITC in monkey LW. This supports the idea that AITC and PITC are reciprocally connected.

The labeled cells posterior to the injection site were not distributed homogeneously but formed clusters. Several patches of labeled cells were observed in V4, in the anterior lip of the lunate sulcus, along the prelunate gyrus, and near the ventral lip of the IOS, and around the TEO/V4 border at the lateral lip of the OTS. The clusters in area V4 were smaller...
We found no labeled cells in the dorsal part of the PIT, where cells had been found to have small RF centered at the fovea. Case 4: Monkey PI

The results of our retrograde tracer injection studies suggested reciprocally connected pathways between the PITC region ventral to the PMTS and the AITC. In these 3 cases, however, few cells if any were observed in the PITC region dorsal to the PMTS, although physiological mapping revealed a cluster of sharply color-tuned neurons in this region (the dorsal color cluster in PITC of LW, Fig. 5B; Yasuda et al. 2010). Where do these color-selective cells in the dorsal PITC region project to?

To address this issue, we injected BDA in the electrophysiologically identified color clusters in dorsal PITC, where neurons have sharp color selectivity and RFs centered at the fovea. We recorded 112 visually responsive neurons from PIT of the monkey PI and quantitatively analyzed 71 of them. Because the PMTS was located very ventral in this monkey, the recording chamber ended up as positioned slightly dorsal to the PMTS. Nonetheless, we could identify a region near the ventral edge of the chamber where neurons had sharp color selectivity but only broad shape selectivity and had RFs

Figure 6. Global distribution of labeled cells in monkey LW. (A) Four coronal sections (a–d). Section (a) includes the 2 injection sites. Section (b) shows labeled cells in posterior TE. Section (c) shows labeled cells lateral to the OTS. Section (d) shows labeled cells around the PMTS. Scale bar = 5 mm. (B) Sulcus-unfolded representation of the labeled cell distributions. Four vertical lines represent the 4 coronal sections in (A). Dotted oval at the ventral lip of the PMTS indicates the ventral color cluster in PITC where CTB-Alexa488-labeled cells were observed. Other conventions are the same as in Figure 2D. For CTB-Alexa488, the total cell count was 9022 and the 95th percentile count was 69 cells per unit area. For CTB-Alexa555, the total cell count was 15 302 and the 95th percentile count was 112 cells per unit area. Scale bar = 5 mm.

Table 3

Quantitative analysis of retrogradely labeled neurons in occipitotemporal cortex of monkey LW

<table>
<thead>
<tr>
<th></th>
<th>TEad</th>
<th>TEav</th>
<th>TEd</th>
<th>TEPv</th>
<th>TEo</th>
<th>A36/35</th>
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<tr>
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<tr>
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<tr>
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<td>TEna</td>
<td>FST</td>
<td>PTFd</td>
<td>V4t</td>
<td>V4</td>
<td>V3/V2</td>
<td>Total</td>
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<tr>
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<tr>
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</tbody>
</table>

Note: Conventions as in Table 2.

We found no labeled cells in the dorsal part of the PIT, where cells had been found to have small RF centered at the fovea. The clusters in the AIT cortex (~4–6 mm²).
We injected 1.2 μL of BDA within this identified color cluster in dorsal PITC (Fig. 9C). The size of tracer uptake zone was 350 μm. Distribution of the axon terminals in the occipitotemporal cortex is shown in Figure 10. Remarkably, we found only very few terminations at the posterior end of the AMTS, the site interconnected with the ventral PITC region in other 3 monkeys. The labeled terminals were sparsely distributed at the fundus of the AMTS and around the posterior dorsal end of the AMTS. Posterior to this sparse terminal focus, however, terminations were densely distributed in an elongated band-like field along the IT gyrus. This band-like terminal field was rather continuous, but it could be viewed as 5 overlapping dense patches of ~4 mm² surrounded by sparser zones (Fig 10C arrows). These results are consistent with the connectivity data from our other 3 monkeys and provide complementary data about the projection from the dorsal PITC region. That is, neurons in this region do not make significant projections to the AITC located at the AMTS; instead, they mainly project to more posterior and dorsal regions in the IT gyrus.

After the BDA injection in the color cluster in the dorsal PITC, axon terminals were also observed in areas TEad, TEav, TEp, and TEO in the IT cortex. Terminations were not observed in TEpv. In the STS, we found labeled axon terminals in areas TEm, TEa, PITd, and IP. Posterior to the injection site, terminations occurred in areas V1, V2, V3 (both V3v and V3d), and V4. In V1, axons terminated preferentially in layer 1. In areas V2, V3, and V4, labeled terminals were mainly in layer 1 but occasionally in layers 2/3 and infragranular layers. In area TEO, very dense terminations in all layers were observed extending anterior to the injection site, but even within area TEO, the terminations could be seen as more prominent in layer 4. In area TE, terminations were dominant in layer 4 but occasionally extended into layers 2/3. We also found labeled terminals in the lateral bank of the intraparietal sulcus and in the subcortical structures such as pulvinar, tail of the caudate nucleus, superior colliculus, and claustrum. These terminal field distributions were generally consistent with previous reports (Webster et al. 1991; Distler et al. 1993; Rockland et al. 1994; Suzuki et al. 2000).

Figure 7. The electrophysiological mapping in PIT cortex and the tracer injection site of monkey KS. (A) Recording sites and the distribution of RFs in the PIT cortex. The arrow indicates the injection site. Other conventions are the same as in Figure 5A. (B) The distribution of SPIs. Conventions are the same as in Figure 4B. (C) Photograph of CTB-Alexa488 injection site (0.5 μL) in the color cluster of the PIT cortex. Scale bar = 3 mm.

centered at the fovea (Fig. 9A,B). These properties indicate that this color cluster is located within the dorsal part of the PITC.

Discussion

Accumulating evidence from lesion studies (Horel 1994; Heywood et al. 1995; Buckley et al. 1997; Huxlin et al. 2000; Cowey et al. 2001) and electrophysiological studies (Komatsu et al. 1992; Koida and Komatsu 2007; Matsumora et al. 2008) has suggested the importance of AITC for color vision but less is known about the inputs to this region. Accordingly, we conducted a series of tracer injection experiments combined with electrophysiological mappings. Our results in fact revealed several well-localized clusters of labeled cells in the region of the posterior occipitotemporal cortex, STS, and prefrontal and parietal cortices. Areas in the posterior TE, regions within STS, and areas TEO, V4, and V2/V3 are candidates for the source of visual signals to AITC. Of these, areas in the posterior TE including the projection patch in STS contained the largest number of labeled cells and, on this basis, are likely the major source of visual inputs (Tables 2 and 3). Area TEO that includes PITC contained the next most substantial number of labeled cells (40-50% of the rest). In the following, we will focus our discussion on the connections between AITC and PITC.
Direct comparison with electrophysiological mapping clearly showed overlap between the distribution of labeled cells and PITC, where another cluster of color-selective cells has been recently reported (Yasuda et al. 2010). From this, we conclude that the PITC is sending a color-selective signal to the AITC. By contrast, in the AIT region outside of AITC, projections originated from subregions in the posterior occipitotemporal cortex distinct from those projecting to the AITC. This result indicates a strong segregation of the projections from PIT to these neighboring AIT regions. Because the cells in these 2 AIT regions clearly differ in their stimulus selectivity, the nonoverlapping projection zones in PIT might reflect a feature-specific projection from PIT to AIT, as suggested previously (Saleem et al. 1993; Moeller et al. 2008). The following sections evaluate the results of our tracer injection experiments in relation to the color-selective regions of the occipitotemporal cortex.

**Projection from PITC to AITC**

The results of our tracer injection experiments clearly demonstrate a projection from PITC to AITC and that the projection patterns of dorsal and ventral regions of PITC are markedly different. Only the cells in the ventral region of PITC...
project substantially to the AITC. Retrograde tracer injections in the electrophysiologically identified AITC resulted in clusters of labeled cells at the ventral lip of the PMTS that coincided with the ventral region of electrophysiologically mapped PITC (case 2). In our experiments, we provisionally defined color clusters as subregions within AITC or PITC, where sharply color-selective but broadly shape-selective cells are accumulated, and the injections were placed in these color clusters. The feedforward projection to the color cluster in AITC mainly arose from the color cluster identified in the ventral PITC. The size of the AITC cluster (~9 mm²) was comparable to the extent of the global terminal field in AIT observed after small anterograde tracer injection in PIT (8.6--9.4 mm² in Suzuki et al. 2000 and ~4 mm² in our case 4). This might suggest that the feedforward projection connects color clusters identified within AITC and PITC in a very specific manner.

By contrast, cells in the dorsal PITC strongly project to the AIT region more posterior and dorsal to the AITC (case 4). So far, no systematic examination of color selectivity has been carried out in this AIT region more posterior and dorsal to AITC, although one imaging study has reported color-selective activation in this region (Tootell et al. 2004). This might suggest another, parallel color-processing pathway involving the dorsal region of the PITC, to be confirmed by systematic mapping of the response selectivity of neurons in this AIT region.

The ventral and dorsal regions of the PIT probably differ in their functions as well as in their projection pattern. Functional heterogeneity of the PIT cortex, including PITC, has been previously suggested (Kobatake and Tanaka 1994; Yasuda et al. 2010). In some support for this idea, our quantitative analysis of projecting neurons showed a functionally heterogeneous distribution of cells in the PITC. Furthermore, RFs are clearly different among the cells in the dorsal and ventral regions of the PITC. In the dorsal region of PITC, neurons have relatively small RFs centered at the fovea, whereas in the ventral region, neurons have larger RFs including both the central and the peripheral visual fields.

Tanaka and colleagues have extensively studied the selectivity of neurons in the IT gyrus that likely include the terminal field of the projection from dorsal PITC and have reported the presence of neurons there that selectively responded to the combination of shape and color (Tanaka et al. 1991; Kobatake and Tanaka 1994). On the other hand, neurons in AITC are selective for color but are relatively insensitive to shape. Therefore, one possible difference between the 2 systems, one including dorsal PITC and the other including ventral PITC, is that the former system is related to the integration of shape and color information, whereas the latter system is related to carry color signals independently (Komatsu and Ideura 1993; Edwards et al. 2003; McMahon and Olson 2009). However, further research is clearly needed to make firm conclusions and more detailed electrophysiological studies might reveal other differences in how color and/or shape processing is carried out.

**Projection from AITC to PITC**

Retrograde tracer injections in the identified color cluster in the ventral PITC revealed a cluster of labeled cells just posterior and dorsal to the AMTS. Although we did not map the AITC in this PITC injection case (case 3), the location of the labeled cell cluster closely corresponded to that of AITC. Combined with results from the injections in AITC, this result strongly suggests a reciprocal connectivity between AITC and the ventral region of the PITC. Interestingly, the extent of the labeled cell cluster in AIT was ~6 mm² comparable to the size of the AITC cluster identified in monkey LW.

The feedback projection from AITC to the PITC can be presumed to be less specific than the feedforward projection from PITC to AITC. Feedback projections have been generally described as divergent (Krubitzer and Kaas 1989; Shipp and Zeki 1989a, 1989b; Rockland et al. 1994), and the feedback projections from AIT to PIT have also been established as more divergent than the corresponding feedforward projections (Suzuki et al. 2000). Consistent with these observations, the projection terminal field in PIT (11.3-20.6 mm² according to Suzuki et al. 2000) is much larger than the color clusters identified in PITC (~4-6 mm²). The PITC clusters are subregions in the PITC and are surrounded by color-selective...
neurons having both color and shape selectivity (i.e., shaded regions in Fig. 5B). The activities of these sharply color- and shape-tuned cells in PITC are potentially modulated by divergent feedback projections from the AITC, and this feedback modulation may play an important role for binding of shape and color (Duncan et al. 1997; Shipp et al. 2009).

Relation with Color-Selective Modules in V4 and STS

Our injections in the color modules of the IT cortex revealed label in sites comparable to those reported in previous anatomical studies in the IT cortex (Morel and Bullier 1990; Webster et al. 1991, 1994; Distler et al. 1993; Borra et al. 2010). While not all these cortical sites are necessarily involved in color processing, some of the sites correspond to color-related regions revealed by recent imaging studies (Tootell et al. 2004; Conway et al. 2007; Harada et al. 2009). Since V4 is considered to be involved in the color processing (Zeki 1973, 1980, 1983; Van Essen and Zeki 1978) and massively projects to PIT (Morel and Bullier 1990; Felleman and Van Essen 1991; Felleman et al. 1997; Ungerleider et al. 2008), the results of retrograde tracer injection in the PITC (case 3) are particularly interesting. On the one hand, the total number of labeled cells was small, and

Figure 10. The distribution of labeled terminals in the occipitotemporal cortex of monkey Pr. (A) Four cropped coronal sections (a-d) at the levels indicated by labeled lines through the schematic brain diagram. In coronal section (a), patchy terminals are sparsely present in the IT gyrus lateral to the AMTS. In section (b), a dense terminal field is evident in a region posterior and dorsal to the AMTS. In coronal section (c), a field of patchy terminals is evident along the IT gyrus. Coronal section (d) includes the injection site and labeled terminals ventrally adjacent and in the lower bank of the STS. (B) Micrographs showing the 3 categories of terminal density. Scale bar = 50 μm. (C) Sulcus-unfolded representation of the distribution of labeled terminals. The density of labeled terminals in 500 × 500 μm² regions is subjectively evaluated and classified into the 3 density levels indicated in (B). Validity of this classification was confirmed by counting the number of boutons of labeled terminals (Supplementary Fig. 5). The density levels are represented as a gray scale. The darkest gray indicates dense axon terminal, the lightest gray indicates sparse axon terminal, and the intermediate gray indicates intermediate density of terminations. The injection site is indicated by a white circle. Black lines indicate the edge of the main sulci, and the broken lines indicate the fundi of the sulci. Scale bar = 5 mm.
the distribution of the labeled cells was rather limited compared with previous reports (Webster et al. 1991, 1994; Distler et al. 1993). This is probably due to our small injection size. On the other, we observed clear patches of labeled cells widely distributed in area V4. One possible explanation for this distribution is the relationship with the visual field representation. That is, the cells in our ventral PITC injection site had relatively large RFs including both fovea and periphery, and the labeled cells were in fact observed in what corresponded to both the foveal and the upper and lower visual field representing region of V4 (Gattass et al. 1988; Ungerleider et al. 2008). Another possibility more important explanation of this distribution is that patchy clusters correspond to sites where color-related activity has been revealed by functional magnetic resonance imaging (Conway et al. 2007) and/or single-unit recordings (Zeki 1973; Kosunoki et al. 2006; Katak et al. 2009). Although this needs to be further examined, these results provide evidence that the ventral PITC region receives converging inputs from color-selective V4 regions representing both the central and the peripheral visual fields.

Notably, our injections of retrograde tracers in either AITC (cases 1 and 2) or ventral PITC (case 3) did not result in significant labeling in PITd, despite the presence there of foci activated by color stimuli (Conway and Tsao 2006; Conway et al. 2007). However, the BDA injection in the dorsal PITC (case 4) showed relatively strong labeling in the lower bank of the STS, likely to be PITd, consistent with Distler et al. (1993). This is another indication that the AITC-ventral PITC connectivity may be distinct from the connection system, which includes dorsal PITC and PITd. An alternative possibility is that PITd is indirectly connected with AITC through the ventral bank of the STS. Our injections in the AITC revealed a prominent projection zone in the lower bank of the STS slightly anterior to PITd (area TEm/TEa). If the color-selective cells in PITd project to this region in the lower bank of the STS, color signals conveyed by the PITd neurons might be indirectly relayed to the AITC.

Conclusions
We have demonstrated reciprocal connectivity between AITC and PITC and distinct projection patterns between the dorsal and ventral regions of the PITC. Strong connections between AITC and the ventral region of the PITC may subserve color processing. By contrast, the dorsal PITC projects to an AIT region slightly posterior and dorsal to the AITC likely to correspond to the region where an imaging study has reported color-related activity (Tootell et al. 2004). Other than PITC, the AITC also receives input from regions in posterior occipitotemporal cortex, STS, and prefrontal and parietal cortices. Not all these regions are necessarily related to color processing, but some are likely to correspond at least partially to sites where previous imaging and unit studies have reported color-related responses (Tootell et al. 2004; Conway et al. 2007; Harada et al. 2009). These observations suggest anatomically interconnected color-processing modules other than the ones we have demonstrated, involving AITC and ventral PITC. The complete network of color modules in IT cortex remains to be determined.

Supplementary Material
Supplementary material can be found at: http://www.cercor.oxfordjournals.org/.

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References
Anatomical Network for Color Vision


