Intrinsic Connections in the Macaque Inferior Temporal Cortex

ICHIRO FUJITA AND TAEKO FUJITA
Precursory Research for Embryonic Science and Technology (PRESTO), Research Development Corporation of Japan, Japan (I.F.); Laboratory for Neural Information Processing, Frontier Research Program, The Institute of Physical and Chemical Research (RIKEN), Wako, Saitama 351-01, Japan (I.F., T.F.); Department of Cognitive Neuroscience, Osaka University Medical School (I.F.), Suita, Osaka 565, Japan

ABSTRACT

Intrinsic connections in the inferior temporal cortex were analyzed by making extracellular injections of biocytin in Japanese macaques. Analysis was focused mainly on the dorsal part of area TE, in which a functional columnar organization has been shown. Interlaminar connections were analyzed in coronal sections after laminar-specific microinjections, and intralaminar connections were examined from tangential sections.

After injections at various depths in the dorsal TE, both axons and cell bodies were strongly labeled above or below the injection site in a columnar appearance. Axons from layer 3 ran in bundles towards the white matter and gave off prominent collaterals in layer 5. Ascending axons from lower to upper layers were also present (e.g., layers 4, 5, and 6 to layer 3). In tangential sections, there were abundant axons running parallel to the pia mater. These horizontal axons, particularly those in layers 2 and 3, produced patches of terminals 0.5 ± 0.1 mm (mean ± s.d.) in size and cylindrical in shape, spanning layers 1–3 or even to layers 4 and 5. In the tangential plane, they were distributed in an anisotropic manner around the injection. The farthest patch appeared at 4 mm from the injection site. The center-to-center distance between nearest-neighbor patches was 0.7 ± 0.3 mm. These patches were found only within the dorsal TE and did not extend into the lower bank of the superior temporal sulcus or into the ventral part of area TE. Area TEO, which is a major afferent source to area TE, had axonal patches with spacing similar to those in area TE but with smaller sizes (0.4 ± 0.1 mm).

The results show that intrinsic horizontal axons both in area TE and in area TEO arborize in a patchy manner, as has been reported for several other cortical areas. In area TE, the size and spacing of the terminal patches match those of columns with similar stimulus selectivity. Thus, these patches may be related to the functional modularity in area TE. Vertical connections across layers and cylindrical patches of horizontal axons most likely contribute to the shared stimulus selectivity among cells within a column.

Indexing terms: visual association cortex, biocytin, horizontal axon, columnar organization, object recognition

Cytoarchitectonic area TE of von Bonin and Bailey (1947) occupies the anterior two-thirds of the inferior temporal cortex (IT) in the monkey (Fig. 1). It represents the final or near-final stage of the unimodal visual cortical stream that is essential to object recognition ("ventral visual pathway") and projects to diverse brain areas, including the frontal and perirhinal cortices, the amygdala, and the striatum. Lesions in this area impair the ability to distinguish different shapes and recognize previously viewed objects, but they do not disturb spatial aspects of vision or visually guided action (Gross, 1973; Dean, 1976; Mishkin et al., 1983; Goodale and Milner, 1992). Consistent with this behavioral result, TE neurons selectively respond to visual attributes that are useful for object identification, such as shape, color, texture, and a combination of these (Gross et al., 1972; Desimone et al., 1984; Baylis et al., 1987; Tanaka et al., 1991; Komatsu and Ideura, 1993; Kobatake and Tanaka, 1994). Stimulus configurations that are required for strong activation of most TE cells are more complex than simple spots, bars, or stripes, examples of which include a T shape, a striped square, a colored star, or a face (Fujita, 1993; Ferrett and Oram, 1993; Tanaka, 1993).

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It has recently been shown that TE cells that are selective for similar object features are grouped in patches roughly 0.4 mm across the cortical surface (Fujita et al., 1992). The patches transcend most cortical layers in a columnar form. Adjacent columns respond to quite different features, and stimuli that activate cells in one column do not excite cells in another column. Two or three separate clusters of cells respond to similar stimuli along a tangential penetration, suggesting that multiple columns are selective for similar features (Fujita et al., 1992).

To explore intracolumnar as well as intercolumnar connections, we analyzed local circuitry within the dorsal part of area TE, which is the portion of the IT where functional columns have been demonstrated. Information on this is very scarce, because most previous anatomical studies on the IT have been focused on the afferent and efferent connections. We made extracellular injections of biocytin into various depths of this area, and we examined the distribution of both labeled axons and cell bodies (King et al., 1988; Lachica et al., 1991; Kenan-Vaknin et al., 1992; McDonald, 1992). Trajectories of single axons were reconstructed to supplement the analysis of the gross distribution pattern of labeled fibers. We showed the existence of strong radial connections across layers as well as clustered arborization of horizontal axons. The size and spacing of patches of horizontal axons were consistent with those of columns with similar selectivity, suggesting a relation of these axon patches to the functional columnar organization. Having found patches of horizontal axons in area TE, we extended our analysis to area TEO, the posterior part of the IT that supplies the major cortical input to area TE (Seltzer and Pandya, 1978; Desimone et al., 1980; Saleem et al., 1993). Area TEO also had similar patches of intrinsic horizontal axons. Preliminary results have appeared in abstract form (Fujita et al., 1991; Fujita and Fujita, 1993).

**MATERIALS AND METHODS**

**Animals and surgical procedures**

Eight Japanese monkeys (*Macaca fuscata*) of either sex weighing 4.1–7.7 kg received focal injections of biocytin into the dorsal part of area TE (see Results; Fig. 1). In two monkeys, injections were made into the dorsal part of area TEO as well (Table 1). The animals were given atropine sulfate (0.06–0.09 mg/kg, i.m.) and were anesthetized with ketamine hydrochloride (6.5–9.4 mg/kg, i.m.) followed by pentobarbital sodium (35 mg/kg, i.p.). Antispasmin (trianexamic acid; 13.0–17.9 mg/kg, i.m.) and carbazochrome sodium sulfate (1.3–1.9 mg/kg, i.m.) were preoperatively injected to reduce bleeding during the surgery. After the head was fixed in a stereotaxic apparatus, all procedures were performed under aseptic conditions. Body temperature and electrocardiogram were continuously monitored, and anesthetics were added when necessary. The monkey was warmed by a heating pad throughout surgery. The scalp was cut, and a large part of the temporal muscle and the skull overlaying the temporal lobe were removed to expose the dura. After biocytin was injected according to the method detailed below, the dura and the skull were cleaned with sterilized saline, and the plate of the skull that had been removed earlier was repositioned and fixed to the surrounding skull with acrylic resin. A mixture of antibiotics (chloramphenicol and fradiomycin sulfate), prednisolone, and lidocaine was administered to the wound margin, and the skin was sutured. Antibiotics (pipercillin sodium, 30.0–33.4 mg/kg, i.m.), analgesics (ketoprofen, 1.6–2.2 mg/kg, i.m.), and 5% glucose (20 ml, s.c.) were injected, and the monkey was kept under observation for 2–5 hours before it was returned to its cage. General animal care and all surgical procedures were in accord with guidelines from the Physiological Society of Japan (1988) and from the National Institutes of Health (1985).

**Tracer injections**

For each injection, we made a small slit (0.5–1.0 mm in diameter) in the dura to insert a glass micropipette into the brain. We directed pipettes at desired positions relative to the superior temporal sulcus (sts) and the posterior middle temporal sulcus (pmts), because we could locate the veins running along the two sulci from above the intact dura. Biocytin (Sigma; 4% w/v; in 0.05 M Tris HCl buffer, pH 8.2) was iontophoretically injected through a glass pipette with anodal rectangular pulses (7 seconds on, 7 seconds off). For most of the injections, the tip size was 10–12 μm in outer diameter, and the injection current was +2–7 μA × 3–8 minutes. In the other cases, we used bigger electrodes (tip size, 20–25 μm) and longer current application (7 μA × 20 minutes) to obtain larger injections. The core of smaller injections measured 200 μm × 140 μm on average (see Table 1 for the size of each injection). Injection core was
TABLE I. List of Trace Injections

<table>
<thead>
<tr>
<th>Injection</th>
<th>Area</th>
<th>Layer/depth</th>
<th>Core size (µm)</th>
<th>Section plane</th>
<th>Figure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. BS-9</td>
<td>TE</td>
<td>1–2</td>
<td>290 x 160</td>
<td>Coronal</td>
<td>3, 4A</td>
</tr>
<tr>
<td>2. B9-1</td>
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<td>2–3</td>
<td>270 x 130</td>
<td>Coronal</td>
<td>3</td>
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<tr>
<td>3. BS-8</td>
<td>TE</td>
<td>2–8</td>
<td>320 x 230</td>
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<tr>
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<td>3</td>
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<td>Coronal</td>
<td>4B</td>
</tr>
<tr>
<td>5. BS-2</td>
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<td>130 x 100</td>
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<td>6. BS-3</td>
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<td>7. BS-5</td>
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<tr>
<td>8. BS-4</td>
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<td>110 x 80</td>
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<tr>
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<td>10. BS-9</td>
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<td>140 x 70</td>
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<td>4F, 11, 12</td>
</tr>
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<tr>
<td>17. B1-1</td>
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<td>Tangential</td>
<td>14</td>
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<tr>
<td>18. B1-2</td>
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<tr>
<td>19. B1-1</td>
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<td>20. B1-2</td>
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<td>21. B1-3</td>
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<td>Tangential</td>
<td>15B, 16</td>
</tr>
<tr>
<td>23. B1-3</td>
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<td>520 x 610</td>
<td>Tangential</td>
<td></td>
</tr>
<tr>
<td>24. B1-3</td>
<td>SI</td>
<td>0.8–1</td>
<td>170 x 160</td>
<td>Tangential</td>
<td></td>
</tr>
<tr>
<td>25. B1-4</td>
<td>SI</td>
<td>0.8–1</td>
<td>500 x 500</td>
<td>Tangential</td>
<td></td>
</tr>
<tr>
<td>26. B1-5</td>
<td>SI</td>
<td>0.8–1</td>
<td>200 x 180</td>
<td>Tangential</td>
<td>15A</td>
</tr>
<tr>
<td>27. B1-4</td>
<td>SI</td>
<td>1.5</td>
<td>130 x 100</td>
<td>Tangential</td>
<td></td>
</tr>
</tbody>
</table>

1The layers are shown for coronal sections, and depth of pipette tip location is shown for tangential sections (mm from the pia mater). u. l, upper and lower tier of layers; WM, white matter.
2The injection core is defined as the center of an injection area where cells and fibers could not be recognized due to dark biocytin deposits. —, the core could not be determined due to inadequate deposition of biocytin.
3Figures in which injections are illustrated.

defined as the area at the center of an injection where cells and fibers could not be recognized due to dense biocytin deposits. Two to seven injections were made in one hemisphere, and two adjacent injections were 3–10 mm apart. Only one hemisphere received biocytin injections in each monkey, and the other hemisphere was used for other studies (Fujita et al., 1992; Ito et al., 1994, 1995; Kondo et al., 1994). Injection depths were varied from site to site in order to explore all cortical layers. Nearby injections were made at different depths whenever possible to help in the identification of injection sites in histological sections.

**Histology**

Nineteen to twenty-five hours after the injections, the animals were killed with an overdose of pentobarbital sodium. After intracardiac injection of heparin (1,000 I.U.) and clamping of the descending aorta, the monkeys were transcardially exsanguinated with 1-2 liters of buffered saline (37°C), fixed by 30-minute perfusion with 4 liters of 4% paraformaldehyde, and finally perfused with 2 liters of 10% and 1 liter of 20% buffered sucrose. Brains were removed from the skull, photographed, blocked, and infiltrated with 30% buffered sucrose solution for cryoprotection. Injection sites were identified with reference to a sketch made during the surgery and plotted on photographs of the brain. Serial frozen sections were cut at 50 µm thickness. Four hemispheres were cut coronally, and the other four were cut tangentially. For tangential sectioning, the IT was dissected from the rest of the brain, and the surface was flattened by pressing it against aluminum foil-covered dry ice. All sections were kept in absolute serial order for reconstruction of single axon trajectories. They were incubated in Vectastain ABC solution (Vector) with 0.4% Triton X-100 for 4 hours at 23°C or overnight at 4°C, rinsed with 0.1 M phosphate buffer, and reacted with diaminobenzidine hydrochloride (DAB) in the presence of 0.01% H2O2. The reaction product was intensified by adding nickel-ammonium sulfate into the DAB solution in a final concentration of 0.03%. Nissl staining was made in selected sections to identify laminar organization (see Fig. 2).

**Data analysis**

Gross distribution pattern of labeled axons was drawn by using ×20 objectives of a microscope (Nikon Optiphot 2). Because multiple injections were made in one IT, particular attention was paid to trajectories of axons to avoid misassignment of labels to a wrong injection site. For observation and reconstruction of single axons, we used ×60 objectives. Distribution of labeled cells was plotted by using ×10 objectives. The identification of labeled neurons was checked at higher magnifications when necessary. For the purpose of superposition of serial sections, brain surface contour, blood vessels, and major labeled axons and dendrites were drawn and were used as landmarks to align serial sections. Laminar borders were determined with the aid of Nomarski optics or darkfield illumination (see Results).

**RESULTS**

We will first describe area parcellation of the IT and the laminar organization of the dorsal TE. We then describe the series with laminar-specific microinjections of biocytin (prepared in the coronal plane). For injections in each layer, we give separate descriptions for labeled axons and cell bodies. Finally, we will describe the organization of labeling across the cortical surface as viewed in the tangential plane of section. This second series includes material from area TEO as well as area TE.

**Area boundary and layer organization**

The IT is not homogeneous across the area in its cytoarchitecture, afferent and efferent connections, receptive field size and stimulus selectivities of cells, and behavioral effects of lesion. Several different schemes have been proposed for area organization of the macaque IT (see, e.g., von Bonin and Bailey, 1947; Seltzer and Pandya, 1978; Turner et al., 1980; Iwai and Yukie 1988; Fellman and Van Essen, 1991). In this study, we followed the parcellation scheme of von Bonin and Bailey (1947), as modified by Iwai and Yukie (1988). In their scheme, the anterior and posterior middle temporal sulci (ants and pmts) are used as landmarks to divide the IT into subareas. Areas TEO and TE are caudal and rostral to the anterior tip of the pmts, respectively (Fig. 1). Area TE is further divided into dorsal and ventral subdivisions (TED and TEv) by the ants. The parcellation is based on simple and unambiguous criterion (i.e., position of sulcus) and is consistent with several lines of evidence from anatomical (Iwai and Yukie, 1987; Martin-Elkins and Horel, 1992; Yukie et al., 1992; Webster et al., 1993), behavioral (Iwai and Mishkin, 1968; Horel et al., 1987; Yaginuma, 1990), and physiological (Boussadou et al., 1991; Tanaka et al., 1991; Kobatake and Tanaka, 1994) studies. Most of our injection sites were made in area TED (Fig. 1, stippled area). For comparison, we made some injections into the dorsal part of area TEO. The injections were limited to the gyrus of these cortices and avoided the lower bank of the stes.
Identification of layers was based on the description by Seltzer and Pandya (1978). Figure 2 shows coronal sections through area TE at the level indicated in Figure 1. Immediately below layer 1 is layer 2, a thin layer of densely packed small cell bodies. Cells near the border between layers 1 and 2 are particularly small and are darkly stained. Cells in layer 3 are bigger and more loosely packed than those in layer 2. The border between layers 2 and 3 is not discrete, yet it can be reasonably delineated on the basis of cell size and density. The size of layer 3 cells increases gradually with the increase in depth of the layer. Layer 4 is the most densely packed layer, with the smallest cells, and it is distinct from other layers. The upper tier of layer 5 contains large pyramidal cells, and cell density decreases in the deeper region of layer 5. In biocytin-reacted sections the upper tier of layer 5 appears darker than the lower tier under darkfield illumination (Fig. 2B). Layer 6 is another densely packed cell layer and appears dark under darkfield illumination, which contrasts with the lower tier of layer 5. The Nomarski optics or darkfield illumination allowed us to identify the layer organization in biocytin-reacted sections without counterstaining (Fig. 2B).

**Labeling studied in coronal sections**

Out of 40 injections made in area TE of 8 monkeys, 29 were selected for analysis (Table 1). Twenty-one of the 29 injections were examined in coronal sections. Results of this analysis are summarized in Table 2.

<table>
<thead>
<tr>
<th>TABLE 2. Summary for Vertical Interlaminar Projections</th>
</tr>
</thead>
<tbody>
<tr>
<td>Layers analyzed</td>
</tr>
<tr>
<td>Layers injected</td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>1-2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
</tbody>
</table>

Note: •, Abundant labels; +, moderate labels; •, inconsistent (present in some cases, but not in others) or weak labels; •, not detected; •, injection layers.

1The strong labeling of axon terminals shown in Figure 3 is interpreted as a result of involvement of cells in the top of layer 3 (see text for details).

**Labeled axons.** Fascicles of axons descended from the injection site. Due to massive labeling of upper layer 3 cells and their dendrites, it was not clear whether these axons exhibited terminal arborization in layer 3 (Fig. 3A). The descending axons extended to layer 4, giving off very few branches or arborizations in this layer (Fig. 3B). In the upper part of layer 5, a cloud of labeled processes spread around the bundles of descending axons. This cloud of labels included branches and terminal arborization of the descending axons as well as dendrites of labeled layer 5 cells. Far fewer axon branches were observed in layer 6 compared to layer 5 (Fig. 3B). Most of the cells of origin of the terminal arborization in layer 5 are likely to be cells in layer 3, but not cells in layers 2 and 4, because fascicles of
descending axons and terminal arborization in layer 5 were not seen in the case where the injection was confined to layer 2 and where only a small number of layer 3 cells were labeled. This interpretation is also supported by an observation that no or few cells were labeled in layers 2 and 4 after layer 5 injections, as shown below (Fig. 4E).

There were also axons running roughly parallel to the pial surface. In layers 1 and 2, a dense labeling of axons surrounded the injection site (Fig. 3A). Some of these axons traversed horizontally in these layers and could be traced up to 4 mm from the injection site. Layers 3 and 5 also contained horizontal axons. Patches of weak arborization were found lateral to the injection site. The patches spanned layers 1 and 2 and the uppermost tier of layer 3. For the injection shown in Figure 3, two such patches were found 1.6 mm and 3.4 mm lateral to the injection site (data not shown). It was not clear whether axons in layers 1 and 2 contributed to these patches, although some axons could be traced back to their origin in layer 3.

Labeled cells. After an injection that spanned layers 1 and 2, the majority of labeled cells appeared around and below the injection site in layers 2 and 3 (Fig. 4A). A small number of cells were also found in layers 4–6 in vertical alignment with the injection site. Retrogradely labeled cells in layer 4 were found in the two injection cases, but they were generally lightly stained. The labeling of layer 4 cells could have occurred because the injections may have involved the top of layer 3. Some cells were scattered up to 770 μm lateral to the injection site in layers 2 and 3.

Layer 3 injections. We made a total of ten injections in layer 3; seven injections confined to layer 3, one injection spanning layer 2 and the upper tier of layer 3, and two injections spanning the lower tier of layer 3 and the upper tier of layer 4 (Table 1). Figure 5 shows a micrograph of an injection in the upper tier of layer 3.

Labeled axons. After layer 3 injections, dendrites of layer 2 and 3 cells were strongly labeled in layers 1 and 2. It was unclear whether axons ascended from layer 3 to layers 1 and 2, although we could observe axon collaterals ascending into layer 2 immediately outside the bulk of labeled dendrites. Fascicles of axons descended through layers 4–6, gave off branches and formed rich terminal plexuses in layer 5, and went out into the white matter (Fig. 5). Weak axon branching was observed in layer 6 after some injections.

Horizontal axons were particularly prominent after layer 3 injections (Fig. 5, arrowheads). They were found most frequently in layer 3 and also less frequently in all other layers. These horizontal axons arborized in a patchy manner at intervals. Figure 6 shows an example of distribution
Fig. 4. Distribution of labeled cell bodies after biocytin injections into layers 1-2 (A), 3 (B), 4 (C), 2-3 (D), 5 (E), and 6 (F) in area TE. Labeled cells are shown as black dots, and the injection sites are indicated by diagonally hatched areas (reconstruction made from 10–20 serial sections). The arrows in D indicate a cluster of labeled cells found lateral to the injection made in layers 2 and 3. An arrow in E indicates a cell that was labeled lateral to the injection to layer 5.
Retrogradely labeled cell bodies were often found in patches lateral to the injection site, but axons of these cells were faintly labeled or only the initial portion of the axons was visible, suggesting that the axonal arbors belonged to the horizontal axons traveling from the injection site and not to recurrent collaterals of the retrogradely labeled cells. Single-axon tracing confirmed this. Figure 7 shows partial reconstruction of two single axons within an axonal terminal patch labeled after an injection into the lower tier of layer 3 (Fig. 7, inset). The patch covered layers 1–5 (Fig. 7, area enclosed by dashed line). Both axons arose from the injection site, ran horizontally or obliquely, and gave off branches to join the patch. Axon 1 entered layer 4 and gave off two branches, one directed upwards (Fig. 7, arrow) and the other descending into layer 5 (Fig. 7, arrowhead). It also made terminal arborization within layer 4 (Fig. 7, asterisk). This suggests that single axons can send signals to more than one layer within a patch. Axon 2 gave off branches only in layer 3 and did not contribute to the lower part of the terminal patch.

Labeled cells. Layers 2–6 contained labeled cells, most of which were in vertical alignment with the injection site (Figs. 4B,D, 5). In contrast to labeling after layer 1-2 injections, a large number of cells were labeled in layer 4 in all but one injection into layer 3. In the lone exceptional case, only two cells were labeled in layer 4, and layers 5 and 6 did not contain labeled cells. It seems that biocytin was not sufficiently taken up in this injection case, because, in all other cases, layers 5 and 6 contained labeled cells. The injections also labeled cells in layers 2 and 3 scattered lateral to the injection site, particularly when injections were large. These cells tend to form clusters (Fig. 4D, arrows). The labeled cells showed pyramidal cell-type morphology when they were well labeled: They had well-differentiated apical and basal dendrites with spines.

Layer 4 injections. We made two injections that were confined to layer 4 (Table 1).

Labeled axons. Figure 8 shows an example of the layer 4 injections. From the injection site, apical dendrites extended towards the upper layers. These dendrites originated from cells in the lower tier of layer 3 and in layers 4 and 5. Many ascending axons with branches and boutons were also observed along or immediately beside these dendrites and were mostly localized in layer 3 (Fig. 8A). There were no fascicles of descending axons similar to those seen after injections into layer 3, except for some axons from labeled cells in layer 5 (compare Fig. 8 to Figs. 3B, 5).

Axons with synaptic boutons were found in layer 5, but it was not clear whether these axons originated from layer 4 cells or layer 5 cells.

Horizontally oriented axons were found within layer 4. These axons arborized either within layer 4 (Fig. 8A, asterisk) or ascended vertically or obliquely into the upper layers and arborized there (Fig. 8B, asterisk). This observation prompted us to perform single-axon tracing to verify whether the axon arbors in layer 3 truly belonged to layer 4 cells or were from retrogradely labeled cells in other layers. Figure 9 shows an example of single-axon reconstruction, where we could trace the axon from its terminals to the cell of origin. The cell was indeed found within layer 4 (Fig. 9, arrow). The cell had a spherical soma and two processes (Fig. 9, inset). One short process was directed downward, and the other long one projected horizontally. This latter process bifurcated 100 μm from the soma. It was not clear whether this process was an axon or a dendrite at this site. Two daughter processes continued to traverse within layer

Fig. 5. A photomicrograph of an injection made to layer 3 in area TE. Some of the thick axons running parallel to the pia mater are labeled by arrowheads. Scale bar = 250 μm.

patterns of anterogradely labeled axons. On a two-dimensional plane reconstructed from 17 serial sections (Fig. 6A), five patches of axon branches and terminals appeared lateral to the injection site at 0.7–1.8 mm center-to-center intervals. Cross-sectional width ranged from 250 to 450 μm. Each patch appeared only on several sections, which was consistent with mediolateral cross-sectional width. The patches, thus, were discrete, and they did not form stripes (see also the results from tangential sections, below). The patches had a cylindrical shape, usually covering layers 1–3, but some patches extended into the infragranular layer. Figure 6B shows such an example in which the arborization was densest in layer 3 but covered layers 1, 2, 4, and 5 as well. Boutons were found most numerously in layer 3 (Fig. 6C). Even layer 6 immediately below this cylindrical cluster contained labeled fibers with terminal boutons. The width of this patch measured 420 μm at the middle of layer 3.
Fig. 6. Distribution of anterogradely labeled fibers after a biocytin injection into layer 3 in area TE (A). Labeling was reconstructed from 17 serial sections covering 850 µm thickness and was projected onto a section that contained the injection site. An asterisk marks the injection site, and arrowheads indicate terminal patches. A patch marked by double arrowheads is drawn in B from a single section. Boutons in the same section are depicted in C. LGN, lateral geniculate nucleus; H, hippocampus.

4. Both gave off branches at almost right angles towards layer 3 (Fig. 9, arrowheads). These branches took a typical axonal morphology in their branching patterns and associated boutons. They ramified in layer 3, and one of the branches ascended farther and reached layer 1. The trajectory of this axon was tilted, but it actually paralleled the direction of radial arrangement of cells and dendrites in the gray matter.

Labeled cells. Figure 4C shows the distribution of labeled cells after the injection shown in Figure 8. This biocytin injection resulted in labeling of cells distributed from the uppermost tier of layer 2 to the lower tier of layer 6. In the other case, cells were found from layer 3 to the lower tier of layer 6. In both cases, most of the labeled cells were in vertical alignment with the injection site. In Figure 4C, the array of cells was tilted, but this was because radial arrangement of cells was tilted at this site near a bend of the cortex. In the other injection into layer 4, one or a few cells were found laterally displaced in layers 3 and 5 (data not shown).

Layer 3 injections. Although we made three injections into layer 5, all of them happened to be smaller than those placed in other layers (Table 1). The injection cores of the two larger injections measured 90 × 70 µm and 80 × 40 µm. In the other case, individual cells and fibers were visible even at the center of the injection, and the core could not be delineated.

Labeled axons. Apical dendrites of layer 5 cells were directed towards the upper layer, with some reaching layer 1 (Fig. 10). Because of the relatively few numbers of dendrites (due to the small injections), we could trace axons intermingled with or running immediately beside the dendrites. Some axons ran directly upwards to layers 3 and 4, whereas others ran slightly oblique to layer 3. Terminal branches and boutons were observed in layer 3. These axons are likely to be from layer 5 cells and not from layer 4.
cells, because layer 4 cells were barely labeled after these injections (Fig. 4E; see below). Axons reached layer 2 in one case, but not in other cases. In all cases, we could not detect axons in layer 1. In layer 6, some axon branchings were observed. Horizontal axons were prominent. Many horizontal axons were labeled even with a small injection in this layer. In the injection shown in Figure 10, several axons ran horizontally in both directions and could be followed up to 2 mm from the injection site. Only a few weak branchings or ramifications of these axons were observed in layer 5, and no clustered patches or ascending branches were found in any of the three injections (see Discussion). Labeled cells. Most labeled cells were found in layers 5 and 6, but there were also a small number of labeled cells in layer 3. In one injection case, a few labeled cells were also found in layer 2. Most of these labeled cells in layers 2, 3, 5, and 6 were found around or in vertical alignment with the injection site (Fig. 4E). Labeling largely avoided layer 4: No cell was labeled after two injections, and a few layer 4 cells were labeled in one injection. In the example shown in Figure 4E, there was a cell 1.85 mm lateral to the injection site at the border between layers 4 and 5 (Fig. 4E, arrow).

Layer 6 injections. Labeled axons. The injection shown in Figures 11 and 12 was made at a bend of the cortex near the ams. It is probably because of this that the labeling spread more laterally than other injections (Fig. 11). However, the densest plexus of labeled axons appeared above the injection site through layers 1–5, mainly in layers 3–5. Of particular note was a cloud of labeling in layer 1 in vertical alignment with the injection site (Fig. 11A, arrow). Although the cloud might include fine tips of dendrites ascending either from the injection site or from labeled cells in layers 3 and 5 (cf. Fig. 4F), there were genuine anterogradely labeled axons. Many axons ran horizontally or obliquely in layers 4, 6, and 6 (Fig. 12). Some of them turned upward or gave an ascending branch and formed clusters in layers 1–3 (Fig. 12A, asterisks). An axon cluster marked with double asterisks in Figure 12A was reconstructed from serial sections and is shown in Figure 12B. The cluster showed a columnar shape spanning layers 1–5, with the densest arborization in layers 1 and 3. The columnar clusters originated from the horizontal axons (some of them marked by arrows in Fig. 12A,B), which could be traced back to the injection site in layer 6. Thus, the anterograde label of these clusters was from layer 6 and not from uptake in layer 5.

Labeled cells. After layer 6 injections, most labeled cells appeared in the lower part of layer 3 as well as layers 5 and 6. Layer 4 cells were not labeled. In the example shown in Figure 4F, no cell was labeled in layer 2, whereas a few layer 2 cells were labeled in vertical alignment with the injection site in the other case. A small number of horizontally displaced, labeled cells were observed in layers 3 and 5.

Labeled cells in the white matter. Some injections led to labeling of cells in the white matter. An example after layer 6 injection is shown in Figure 11 (arrowheads). These cells were located in the white matter, as evidenced in the darkfield micrograph ("interstitial cells": Fig. 11B). The somata were spherical, irregular, or pyramidal in shape. The soma diameter of these cells was 12.6 (±2.5) × 9.2 (±1.6) μm (mean ± s.d.; n = 28). The cells had a long ascending dendrite and a spray of short basal dendrites. The ascending dendrites coursed into the gray matter in an antiparallel manner with descending axons from the injection site (Fig. 11C). The dendritic shaft was equipped with numerous spines (Fig. 11D). This dendritic morphology is quite different from NADPH-positive interstitial cells, which are typically multipolar (Fujita and Fujita, unpublished observations).

Tangential distribution of labels

We analyzed eleven injections in tangential plane sections; eight injections in area TE, two in area TEO, and one in a transition area between the TEO and the TE. Organization of anterograde labeling. In tangential sections, the injection core was surrounded by a dense labeling of radiating horizontal axons (see Fig. 16). This was observed in all injections made at various depths. In six of the eight injections made into area TE, patches of horizontal axons appeared at varying distances. The number of patches ranged from seven to nearly 20. There was a trend that larger injections produced more patches, and the two smaller injections (Table 1, B1-1 and B1-2) failed to
produce patches. When we traced the patches in serial sections, it became clear that most of the patches spanned many sections (Figs. 13, 14), indicating that the patches had a cylindrical shape that was elongated in the direction of cortical depth. Indeed, at the edge of sections bordering sulci where the section plane was perpendicular rather than tangential to the pial surface, patches were elongated in a bell shape and spanned many layers (Figs. 13, 15B).

The overall distribution of the patches was not radially symmetric around the injection site. In the example shown in Figure 13A, most patches appeared dorsal and ventral to the injection site, whereas, in the example in Figure 13B, all but one patch appeared dorsal to the injection site. In both cases, few patches appeared anterior or posterior to the injection site. On the other hand, in the example shown in Figure 14, most patches were found anterior or posterior to the injection site, with few appearing dorsal or ventral to it. Thus, the patches were distributed in an elongated manner parallel to the sts (Fig. 14, inset). This injection showed the widest spread of patches in our sample; the center-to-center distance between anteriormost and posteriormost patches was 7.35 mm, and that between dorsalmost and ventralmost patches was 4.6 mm (anisotropic ratio of 1.6). The farthest patch was located 4.0 mm from the injection site. In all cases, patches were confined to the dorsal part of area TE. Ventrally, no patch was found beyond the ams in the ventral part of area TE. Dorsally, patches were found along the edge of tangential sections that represented the shoulder of the lower bank of the sts. When we examined injections prepared in coronal sections, horizontal axons were found only up to the shoulder of the sts and did not extend into the lower bank of the sts.

Size and spacing of terminal patches of horizontal axons. We measured diameters along the short axis (width) and the long axis (length) of horizontal axonal patches at a depth where patches were most clearly visible. For this measurement, we selected patches that spanned at least three alternate sections (i.e., patches spanning at least 250 μm), and the measured values were averaged over three sections. We did not include the patches that were found at the edge of sections, because the section plane was vertical, rather than tangential, to the cortex near the edge. The width was 411.7 ± 80.0 μm (mean ± s.d.; 21 patches from three injections), the length was 689.7 ± 145.1 μm, and the average of width and length was 510.7 ± 92.9 μm. The area of patches in area TEd was 0.20 ± 0.08 mm². When we measured center-to-center distance between a patch and all of its surrounding neighbors, the distance ranged from 0.29 to 5.39 mm (1.9 ± 1.1 mm). When the measurement was made only between a patch and its nearest neighbor, the
distance was 0.29–1.56 mm (0.7 ± 0.3 mm). Within an area outlining the outermost patches, the patch densities were 0.75, 0.82, and 1.43 patches/mm² for injections shown in Figures 13A, B, and 14, respectively.

Retrogradely labeled cells. The number of retrogradely labeled cells after biocytin injections varied considerably from case to case. When small numbers of cells were retrogradely labeled, they tended to be found in the patches of anterogradely labeled axons. However, in the cases where many cells were labeled, cells were found both inside and outside the axonal patches (see Discussion). In the reconstruction shown in Figure 14, labeled cells are shown as small black dots. One hundred and ninety cells were found inside patches, and 129 were found outside. Some axonal patches were devoid of any labeled cells inside (e.g., the patch indicated by an asterisk in Fig. 14). This suggests that some of the patches were reciprocally connected with the injection site, but others were not.

Horizontal patches in area TEO. Injections into area TEO also produced labeling of radiating horizontal axons and patches of terminals (Figs. 15, 16). In the example shown in Figure 15A, patches were not found anterointernal to the injection site. In the case in Figure 15B, an injection was made at the border between area TEO and area TE. This injection produced more than 25 patches, which appeared both anterior and posterior to the injection site. The width of the patches was 349.7 ± 79.6 μm, the length was 491.7 ± 118.1 μm, the average of width and length was 420.7 ± 88.8 μm, and the area was 0.13 ± 0.06 mm² (30 patches from the two injections shown in Fig. 15). All of these values are smaller than the counterparts for area TE (P < 0.005 for the width and length, P < 0.001 for the average of width and length, and P < 0.0005 for the area; Student's t-test). The center-to-center distance between a patch and all of its surrounding neighbors ranged from 0.39 to 3.55 mm (1.4 ± 0.7 mm; n = 85). The distance to the nearest neighbor from each patch ranged from 0.39 to 1.42 mm (0.8 ± 0.3 mm; n = 30) and did not differ from that measured in area TE. The patch density was 0.95 patches/mm² for the injection in Figure 15A, and 1.15 patches/mm² for the injection in Figure 15B.

DISCUSSION

Biocytin injections labeled two major groups of axons in area TE; one running along the radial arrangement of cells in the cortex and the other running mostly parallel to the pial surface. Labeled cell bodies also appeared both in
Fig. 11. Photomicrographs of labeling after a biocytin injection into layer 6 in area TE. Brightfield (A) and darkfield (B) photographs of the same section through the injection site. Because the reaction product was nickel intensified, the darkfield illumination does not show a clear image of labeled fibers but helps to identify the layers. A cluster of neurons was labeled in the white matter (arrowheads). Arrow in A denotes a cloud of labeling in layer 1. Nomarski pictures of the labeled cells show ascending dendrites (C) with numerous spines on the surface (D). Scale bars = 250 μm in A, B, 40 μm in C, 20 μm in D.

Fig. 12. Distribution of labeled axons after the layer 6 injection shown in Figure 11. A: Drawing made from a single section adjacent to the section shown in Figure 11. Some horizontal axons ascended from layer 6 to superficial layers to form cylindrical arborization (asterisks). B: Reconstruction of the patch labeled by double asterisks in A made from four serial sections (covering 200 μm thickness). Axons marked by arrows in A and B originated from the injection in layer 6, and not from labeled cells in layer 5.
vertical alignment with and lateral displacement from the injection sites. The horizontal axons gave rise to patches of terminals in area TE as well as in area TEO.

**Technical consideration**

Although extracellular microinjections of biocytin provide a useful means to study local connections in the cortex (see, e.g., Lachica et al., 1992; Yoshioka et al., 1992; Amir et al., 1993), results must be interpreted with the utmost care. For example, columnar labeling of cell bodies after an injection (Fig. 4) does not necessarily imply vertical connections between the injection layer and the labeled layer. Some of the labeling of cell bodies above or beneath the injection site could be due to uptake from passing fibers or from dendrites and not by uptake from axon terminals.

Lachica et al. (1991) reported that biocytin is not taken up by passing fibers. We also did not find any sign of labeling of passing fibers after an inadvertent injection into the white matter 300 μm below the bottom of layer 6 (data not included in this study). Likewise, injections into layer 4 did not label fascicles of axons descending from layer 3 (Fig. 8). These injections, however, were slightly smaller than most of our injections in the present study, and we should leave open the question of biocytin labeling of fibers of passage.

Labeled cells may be interpreted as retrogradely labeled from axon terminals if their dendrites do not reach the injection site. For this reason, labeled cells in layer 3 after injections into layers 5 and 6 might reflect projection from layer 3 to layers 5 and 6, because labeled dendrites of these layer 3 cells did not reach layers 5 and 6. Strong labeling of terminal plexuses in layer 5 after injections into layer 3 confirmed the projection from layer 3 to layer 5. Labeled cells appearing below the injection site are harder to interpret, because, in many cases, these labeled cells extend their apical dendrites into the injection site. Indeed, layers below injection sites almost always contained labeled cells (Table 2), and some of the labeling might be caused by dendritic uptake. For projections from lower to upper layers, anterograde labeling provides more accurate results. Ascending
axons observed in layer 3 after layer 4, 5, or 6 injections indicate vertical projection from these lower layers to the supragranular layer.

Labeled cells found lateral to the injection sites are not affected by the issue of dendritic uptake, because most of them appeared far beyond the dendritic extent of cells at the injection sites. Instead, their presence raises a concern that recurrent collaterals of these retrogradely labeled cells may contribute to the axon patches we described. This is unlikely, however, because axons of these labeled cells are not labeled or are only faintly labeled. Contribution of recurrent collaterals of the retrogradely labeled cells to these axon patches was minimal, if any, in our biocytin-labeled materials.

Smaller injections tend to label a smaller number of patches, as reported by others (Yoshioka et al., 1992; Amir et al., 1993). This might be the reason why we saw more patches in tangential sections, where larger injections were made, than in coronal sections, where smaller injections were made for localization in a single layer. Amir et al. (1993) interpreted their results as evidence for a discontinuous map in which neighboring cortical sites are connected to separated sets of cortical sites in the same area. We are currently applying double-antegrade as well as double-retrograde labeling techniques to area TE in order to test whether neighboring sites in area TE have separated sets of intrinsic input/output connections.

When injections were small, labeled cells were mostly pyramidal cells and tended to localize inside axon patches. Larger injections labeled some nonpyramidal cells in addition to pyramidal cells, and the distribution of both types of neurons did not always coincide with axon patches (Fig. 14; cf. Boyd and Matsubara, 1991). A recent study using fluorescent dyes (fast blue or diamidino yellow) as a retrograde tracer have confirmed that single injections of these dyes label from several to more than 15 columnar clusters of cells as well as cells diffusely scattered between the clusters (Fujita et al., 1995). Intracellular injections of
Lucifer yellow in fixed slices have revealed that most of the cells of origin of horizontal axons are pyramidal cells (standard pyramids, asymmetric pyramids, and inverted pyramids), some are fusiform cells (particularly in the infragranular layer), and a few were classified as nonpyramidal interneurons (Fujita et al., 1995).

**Interlaminar connectivity**

Figure 17 summarizes the local connections in area TE that are suggested by the present results. These results demonstrate extensive vertical interlaminar connections in TE (Fig. 17, left). Cells in layer 3 project heavily to layer 5, whereas cells in the infragranular layer project to some of superficial layers. Axons of layer 4 cells also ascend to layer 3. Many of these layer-to-layer connections are common to area TE and other visual cortical areas. For example, prominent terminal plexuses in layer 5 after injections into layer 3 are found in V1 (Blasdel et al., 1985; Lachica et al., 1992), V4 (Yoshioka et al., 1992), and area TE (this study). The labeling pattern in V4 after injections to individual layers (Fig. 4 in Yoshioka et al., 1992) is basically similar to that found in area TE. However, there exist some differences between cortical areas, particularly between V1 and other areas, probably because of the well-developed layer 4 and structural compartmentalization in V1. One such difference is that an injection to layer 2-3 in V1 results in labeling of dense axon collaterals in layer 4B as well as in layer 5 (Blasdel et al., 1985). In V1, cells within cytochrome oxidase-rich blobs differ from cells outside blobs in their vertical interlaminar connections (Lachica et al., 1992), whereas, in TE, no consistent difference was detected in the labeling pattern among injections made in the same layer. More detailed analysis of interlaminar connections in area TE are beyond the aim of this study and may require more refined techniques, such as intracellular staining of cells.

The present study also showed that the intrinsic horizontal axons in area TE produced patches of terminals 0.5 mm in width, spanning the supragranular layer, and sometimes extending into the infragranular layer (Figs. 6, 7; Fig. 17, right). In addition, Saleem et al. (1993) have recently shown that a single injection of an anterograde tracer (*Phaseolus vulgaris* leucoagglutinin) into area TEO labels a few dense foci of terminal arborization in area TE, each measuring 0.5 mm wide. The axon terminals in these foci are distributed throughout the gray matter. All of these anatomical features, vertical interlaminar connections and cylindrical arborization of intrinsic horizontal axons and afferent fibers, enable extensive interactions across layers with an...
approximate horizontal extent of 0.5 mm and can provide an anatomical basis for functional columns in which cells with similar physiological properties cluster vertically.

**Patches of horizontal axons**

Biocytin injections into layers 2, 3, 4, and 6 labeled patches of horizontal axon arborization in area TE (Figs. 6–9, 12–14). Although biocytin may be taken up only by a certain type of neuron (cf. McDonald, 1992), similar patches were observed after injections of wheat germ agglutinin conjugated to horseradish peroxidase or biotinylated dextran amine into area TE (I. Fujita, unpublished observations). Cells at a given site in area TE, therefore, project their horizontal axons preferentially to particular foci, and they are not diffusely connected with cells at surrounding sites. The axon patches were ovoid or circular in shape, and...
they did not form stripes like those found in the frontal cortex (Lund et al., 1993). Similar patches were found in area TE (Fig. 15) and have recently been shown in the rostral IT of the squirrel monkey (Weller and Steele, 1993; owl monkey: see Fig. 13 of Weller and Kaas, 1987).

Axon patches were not observed after layer 5 injections (Fig. 10). This may be due partly to inadequate deposition of biocytin, because all of our layer 5 injections were smaller than the other injections. On the other hand, the poor labeling of patches after layer 5 injections may be genuine, because horizontal axons of layer 5 pyramidal cells in the cat auditory cortex do not produce a dense columnar patch comparable to that observed in the axons of layer 2-3 pyramidal cells (Ojima et al., 1991, 1992). Injections of biocytin into layer 5 of the macaque V4 also did not label strong terminal patches in the supragranular layer (Yoshioka et al., 1992). Similar to other cortical areas, the patches were not extensively labeled after injections into layers 2 and 3 and appeared in the largest numbers in layer 3 (Fig. 6).

Long-range horizontal axons and their patchy arborizations were first described in area 17 of cats (Gilbert and Wiesel, 1979) and are now known to be present in many cortical areas of diverse mammalian species (LeVay, 1988). In the ventral visual pathway of the macaque cortex, patches of horizontal axons have been described previously in V1, V2, and V4 (Rockland and Lund, 1983; Rockland, 1985; Livingston and Hubel, 1984; Yoshioka et al., 1992; Amir et al., 1993). Thus, horizontal axons and their clustered terminal arborization are found in every major step (V1, V2, V4, TE, TE) of the macaque ventral visual pathway.

The size of axonal patches gradually increases from V1 to V4 (Yoshioka et al., 1992; Lund et al., 1993; Amir et al., 1993). On the basis of biocytin injections that were similar in size to those made in this study, Amir et al. (1993) reported that the patch widths for V1, V2, and V4 are 230, 250, and 270 mm, respectively. Lund et al. (1993) reported the average of width and length as being 230 mm for V1, 340 mm for V2, and 350 mm for V4. Areas of patches are 0.068 mm² in V1, 0.075 mm² in V2, and 0.1 mm² in V4 (Amir et al., 1993). Comparison of these values to the values obtained in this study suggests that patches are even larger in the IT. Furthermore, within the IT, patches in area TE are larger than those in area TEO (area TEO: width 350 mm, length 490 mm, average of width and length 420 mm, and area 0.13 mm²; area TE: width 410 mm, average of width and length 510 mm, and area 0.2 mm²). However, we need to compare the IT and earlier visual cortical areas in the same animal, because these values might be influenced by size and species of monkey, fixation protocols, and the way in which we delineate the patches.

Lund et al. (1993) examined the size of patches in V1, V2, and V4 as well as motor (area 4), somatosensory (areas 3b, 1, 2), and prefrontal cortices (areas 9, 46) and found that the patch size closely coincided with the horizontal extent of basal dendrites of layer 3 pyramidal cells in each area. Although the functional meaning of this coincidence remains to be established, it would be interesting to analyze Golgi-impregnated sections to see whether this rule holds true for areas TEO and TE.

It is difficult to compare the patch distance in the present study to the distances reported for V1, V2, and V4 in previous studies (Amir et al., 1993; Lund et al., 1993), because we are not sure between which patches the measurements were made. There is also a large discrepancy in the reported values of interpatch distances between the two previous studies.

The farthest patch within area TE appeared as far as 4 mm from an injection site. Similarly distant patches have also been reported for other cortices. Intracellular staining of V1 pyramidal cells reveals the presence of intrinsic horizontal axons extending 6–8 mm (Gilbert and Wiesel, 1979). Horizontal axons from injections in the dorsal TE, however, are confined within this area and did not appear in adjacent cortices within the lower bank of the sts or beyond the ams. It is still possible that some of the farthest patches found in tangential sections may represent extrinsic connections between yet-to-be-established subdivisions in the dorsal TE. There is accumulating evidence that area TE is not a single, homogeneous area but consists of a few subareas (Horel et al., 1987; Iwai and Yuki, 1987; Yaginuma, 1990; Martin-Elkins and Horel, 1992; Yuki et al., 1992; see also the Discussion in Weller and Kaas, 1987).

Relation between patches and functional architecture

In area 17 of cat brain (Ts'o et al., 1986; Ts'o and Gilbert, 1988; Gilbert and Wiesel, 1989) and monkey V1 (Malach et al., 1993), intrinsic horizontal axons of layer 2-3 pyramidal cells tend to connect columns with similar orientation selectivity. It is also suggested in area 17 of ferret cortex that horizontal axons link columns that are related not only in their orientation selectivity but also in their visual topography; the terminal patches connect columns distributed across the visual field map along the axis of preferred stimulus orientation (Fitzpatrick et al., 1993). In area 18 of cat brain, on the other hand, it has been suggested that columns selective for orthogonal orientation are interlinked (Matsukura et al., 1987). Regarding the ocular domes of columns, again, we have a discrepancy between reports: one with evidence for preferential link among columns with the preference for the same eye (Ts'o and Gilbert, 1988; Malach et al., 1993) and the other with evidence for connections among columns with same as well as different ocular preferences (Lowel and Singer, 1992). Some of the discrepancies may be related to cellular substrates of horizontal
axons. The horizontally directed axons in cat areas 17 and 18 are emitted not only from pyramidal cells but also from basket cells. The basket cells in layer 3 of cat visual cortex have been shown recently to innervate columns with the complete range of orientation preferences (Kisvárday and Eyssel, 1993).

Lund et al. (1993) also emphasized the relation between the anisotropy of the terminal patch distribution and the anisotropy of the visual field map in V1, V2, and V4. We did find the anisotropy of terminal patch distribution in area TE (Figs. 14, 15), but it is unlikely that this anisotropy is related to the visual field. Receptive fields of TE neurons are large, extending 15–40° or more (Gross et al., 1972; Desimone et al., 1984; Tanaka et al., 1991; Kobatake and Tanaka, 1994) and, in most cases, include the fovea. Area TE has no retinotopic map (Desimone and Gross, 1979). Together, these observations indicate that columns in area TE have receptive fields that largely overlap with others. Horizontal axons in area TE, therefore, connect columns with overlapping receptive fields and are not used in the interactions between neurons responding to different parts of the visual field, as proposed for V1 (see, e.g., Gray et al., 1989; Amir et al., 1993).

We then ask what is the relation between columns connected by horizontal axons in area TE. It is likely that these connections are related to stimulus selectivity of columns concerned with visual features of objects. A particularly interesting question is whether there is a possible relation of the patches to multiple columns with similar stimulus selectivity observed in physiological experiments (Fujita et al., 1992). In a tangential electrode penetration made in area TE, we often find two or three clusters of cells responding to similar object features. These “satellite” columns appear at intervals of 0.4–1.0 mm distance (Fujita et al., 1992). The width of columns measured in physiological experiments is 0.4 ± 0.2 mm, which is comparable to the patch size measured in this study. The value is slightly smaller and more variable than the width of axon patches, but this may be because electrode penetrations do not always pass through the center of columns, whereas the measurement in this study was taken from the largest diameter across each patch. The distance between columns with similar selectivity was also within the range of the distance between the injection sites and surrounding patches of horizontal axons observed in this study. These results suggest a relation between the horizontal axon patches and the functional columnar organization in area TE.

The surface area of the dorsal part of area TE, from which our previous physiological recordings were made, is 330 ± 76 mm² (four hemispheres from two monkeys). The number of functional columns has been roughly estimated at 2,000 (Fujita et al., 1992). If we assume that the horizontal axons link columns with similar stimulus specificity, then we can predict the number of different types of columns simply by dividing this number by the average number of patches of horizontal axons originating from a site. If we take ten as the average patch number, then the number of different types of columns will be 200. This assumption, however, is totally speculative at this moment, and, in order for this calculation to be meaningful, we have to know how functional columns relate to horizontal axonal patches. One way to analyze this relation will be to make simultaneous recordings from neurons in two separated columns and determine stimulus selectivity and cross correlation between the two neurons, as has been done for other cortices (Ts’o et al., 1986; Ts’o and Gilbert, 1988). A combination of optical imaging and anatomical fiber-tracing techniques will be a more favorable, but technically demanding, approach to determine whether horizontal axons link columns with similar or different selectivity or whether a more complicated rule exists (for similar experiments in cat visual cortex, see Malach et al., 1993; Yoshioka et al., 1995).

Anatomical gradients along the ventral visual pathway

Constant increase in the size of the patches of terminals is not the sole change that can be observed along the ventral visual pathway. Other anatomical changes include the following (Gross et al., 1993): progressively less topographic afferent connectivity (Fenestemaker et al., 1986), progressively denser and more widespread callosal connections between both hemispheres (Van Essen et al., 1982), and progressively heavier and more extensive connections with the amygdala (Turner et al., 1980; Iwai and Yukie, 1987). There are also neurochemical gradients along the pathway. Concentration of a subtype (μ-like) of opiate receptors (Lewis et al., 1981), phosphorylation levels of protein kinase C substrates (protein F1 and a 81 kDa protein; Nelson et al., 1987), and the number of calbindin-immunoreactive pyramidal cells (Kondo et al., 1994) gradually increase towards the anterior portion of the pathway. These anatomical and neurochemical aspects may be related to changes in physiological characteristics of neurons, such as increase in receptive field size, complexity of stimulus selectivity, and modulation of neuronal activity by attention and learning, although an understanding of exactly how changes take place and are related to physiology will require more studies.

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LITERATURE CITED
