 ORIGINAL ARTICLE

Postnatal Development of Intrinsic Horizontal Axons in Macaque Inferior Temporal and Primary Visual Cortices

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Abstract

Two distinct areas along the ventral visual stream of monkeys, the primary visual (V1) and inferior temporal (TE) cortices, exhibit different projection patterns of intrinsic horizontal axons with patchy terminal fields in adult animals. The differences between the patches in these 2 areas may reflect differences in cortical representation and processing of visual information. We studied the postnatal development of patches by injecting an anterograde tracer into TE and V1 in monkeys of various ages. At 1 week of age, labeled patches with distribution patterns reminiscent of those in adults were already present in both areas. The labeling intensity of patches decayed exponentially with projection distance in monkeys of all ages in both areas, but this trend was far less evident in TE. The number and extent of patches gradually decreased with age in V1, but not in TE. In V1, axonal and bouton densities increased postnatally only in patches with short projection distances, whereas in TE this density change occurred in patches with various projection distances. Thus, patches with area-specific distribution patterns are formed early in life, and area-specific postnatal developmental processes shape the connectivity of patches into adulthood.

Key words: axonal morphology, column, exponential distance rule, horizontal connections

Introduction

Horizontal axons of pyramidal cells are prominent components of the intrinsic neuronal circuitry of mammalian neocortex. Running parallel to the pial surface for more than several millimeters within a cortical area, they terminate in patchy terminal fields, predominantly in layers 1–3, to connect distant sites (Gilbert and Wiesel 1979, 1983; Rockland and Lund 1983; Martin and Whitteridge 1984; Blasdel et al. 1985). In primary visual cortex (V1), these long-range horizontal axons interconnect cortical sites with similar response properties (Gilbert and Wiesel 1989; Malach et al. 1993; Yoshioka et al. 1996; Bosking et al. 1997). The exact role of horizontal axons in cortical functions remains enigmatic; those in V1 are implicated in formation of orientation maps (Callaway and Katz 1990; Ruthazer and Stryker 1996; Shouval et al. 2000), adaptive changes of cortical maps after deafferentation (Darian-Smith and Gilbert 1994; Das and Gilbert 1995; Yamahachi et al. 2009), and activity synchronization of spatially distant neurons (Gray et al. 1989; Smith and Kohn 2008).

The cytoarchitectonic area TE, located in the anterior part of the macaque inferior temporal cortex (Bonin and Bailey 1947), is one of the later stages of the ventral visual stream. In an effort to elucidate the functional architecture of TE, we compared the organization of horizontal axons in TE with that of V1 (Tanigawa
et al. 2005; Fujita and Fujita 1996). These studies revealed marked differences in the projection patterns of horizontal axons between the 2 areas. The size, spacing, and maximal lateral extent of terminal patches of horizontal axons are greater in TE than in V1. In addition, the anisotropy of the overall tangential distribution of patches originating from a given cortical site is greater in TE than in V1. The axonal density of patches gradually declines as projection distance increases in V1, but this feature is less apparent in TE: some distant patches exhibit stronger labeling than patches with short projections. These differences in horizontal axons may reflect differences in the representation and processing of visual information in the 2 areas; unlike V1, TE exhibits neither a retinotopic nor an ordered map for any particular stimulus parameters (Gross et al. 1972; Desimone and Gross 1979; Fujita et al. 1992; Fize et al. 2003).

How do these area-specific features of horizontal axons develop? In monkey V1, horizontal axons terminate in a patchy manner prenatally, at embryonic day 145 (Coogan and Van Essen 1996). In cat and ferret V1, terminal patches of horizontal axons emerge only postnatally, but prior to eye opening (Callaway and Katz 1990; Luhmann, Singer, et al. 1990; Lübke and Albus 1992; Galuske and Singer 1996; Durack and Katz 1996; Ruthazer and Stryker 1996). These studies indicate that the formation of patchy projections does not require visual experience in V1. Instead, spontaneous activity through geniculocortical projections, which are present before birth, plays a key role in the initial formation of the intracortical connections (Ruthazer and Stryker 1996; Sur and Leamney 2001). In contrast, postnatal refinement of these projections requires visual experience (Callaway and Katz 1991; Ruthazer and Stryker 1996; White et al. 2001). However, the details of such refinement, particularly with respect to the distribution of terminal patches, remains unexplored. Much less is known about the development of horizontal axonal projections in TE. Neurons in TE do not exhibit adult-like responsiveness within the first half-year after birth (Rodman et al. 1991, 1993). Given the lack of direct geniculocortical projections to TE (Sorenson and Rodman 1996), one reasonable speculation is that horizontal axons in TE are immature early in life. However, despite their weaker responsiveness, TE neurons already exhibit adult-like stimulus selectivity, for example, for faces and geometrical patterns, at several months of age (Rodman et al. 1991, 1993), suggesting that a developed intracortical network exists to some extent in infant TE. Therefore, in order to understand the functional role of these projections in TE, it is necessary to elucidate the postnatal development of projections of horizontal axons.

In this study, we analyzed the postnatal development of horizontal axons in TE and V1 through extracellular injections of an anterograde tracer in monkeys of various ages. Our results demonstrate that even 1 week postnatally, horizontal axons exhibit patchy termination with area-specific, adult-like morphological characteristics and distribution patterns in both TE and V1. However, morphology within patches, including axonal and bouton densities, continues to be refined in an area-specific manner, resulting in divergent developmental profiles in the 2 areas.

Materials and Methods

Animals

Twenty-five Japanese monkeys (Macaca fuscata) were used in the present study, ranging in age from 1 week to >3 years (Table 1). We made all injections unilaterally into TE and V1. All animals also participated in other anatomical and/or electrophysiological studies (Uka et al. 1999, 2000; Tanigawa et al. 2005; Xu et al. 2003). A portion of the data presented here was also used for another study examining horizontal axons in TE and V1 of adult monkeys (Tanigawa et al. 2005). Surgical and animal care procedures, approved by the animal experiment committee of Osaka University Medical School, were in accordance with guidelines from the National Institutes of Health.

Surgical Procedures

The surgical procedures for adult monkeys are described elsewhere (Fujita and Fujita 1996; Tanigawa et al. 1998, 2005). The surgical procedures for neonates and infants will be described here in detail. Neonates and infants were deprived of milk or food for 4–12 h prior to surgery. After atropine sulfate (0.04 mg/kg, i.m.) was administered to reduce salivation, initial anesthesia was introduced with ketamine hydrochloride (15 mg/kg, i.m.) and acepromazine (1.5 mg/kg, i.m.). Tranexamic acid and carbazochrome sodium sulfate were given to minimize bleeding, and dexamethasone phosphate was administered to prevent cortical edema. Following the insertion of an endotracheal tube, surgical anesthesia was maintained with isoflurane (1.25–1.5%). After fixing the head in a stereotaxic apparatus, all procedures were performed under aseptic conditions. Body temperature was maintained at 36–38°C by a heating pad. Heart and respiratory rates, expired CO2 levels, and arterial oxygen saturation levels were monitored throughout the surgery.

Following topical application of lidocaine, the scalp was cut, and a part of the temporal muscle was removed. A separate hole with a diameter of 3–4 mm was drilled in the skull for each injection. A small slit (0.5–1.0 mm) was made in the dura. After we completed injections with the procedure described below, the dura was pulled over the cortical surface, cleaned with saline containing an antibiotic, amikacin sulfate, and covered with a piece of gelatin sponge (Spong; Yamamouchi, Tokyo, Japan). The hole in the skull was filled with dental acrylic resin. Following the application of an antibiotic, fradiomycin sulfate, to the wound, the skin was sutured and disinfected with iodine. After cessation of isoflurane, animals were ventilated with oxygen, recovering within 20 min. Infants below 1 month of age were given drops of 50% dextrose on the tongue to aid recovery. Antibiotics and analgesics were administered as described in Tanigawa et al. (1998).

Tracer Injection

Biotinylated dextran amine (BDA; M.W. 3000, Molecular Probes, Eugene, Oregon) was injected by 10 min of iontophoresis (+7 μA in a 7 s ON/OFF cycle). A glass micropipette with a 32–36-μm inner tip diameter, filled with 10% BDA dissolved in 0.01 M phosphate buffer (PB, pH 7.4), was positioned normal to the cortical surface. The tip of the pipette was advanced into the cortex to a depth of 0.75–1.25 mm, then withdrawn to 0.4–0.5 mm for V1 and 0.75 mm for TE to confine the injection to layers 2 and 3. To prevent the labeling from different injections from overlapping, only 1–2 injections were made in TE and 1–4 injections were made in V1 in each monkey.

We determined injection locations by the same criteria as we applied in our previous study (Tanigawa et al. 2005). Briefly, in TE the injections were made into the crown of the middle temporal gyrus. According to the nomenclature proposed by Yukie et al. (1990) and modified by Saleem and Tanaka (1996), the injections were located in TEc, the dorsal part of TE. According to the other classifications, the injections were considered to be located in cytoarchitectonic areas TE2 and TE3 of Seltzer and Pandya (1978), or in CITv and AITv of Felleman and Van Essen (1991). Some injections near the ventral lip of the superior temporal
The brains were removed and photographed, then blocked, postfixed overnight in fixative, and soaked in 10–30% sucrose PBS for cryoprotection. The surface of the blocks of TE and V1 containing the injection sites was first flattened by pressing it against aluminum foil-covered dry ice, and then the whole blocks were frozen with dry ice powder. No physical unfolding of tissues was performed. Serial sections were cut tangentially to the flattened surface on a freezing microtome at 50 µm. Sections were rinsed with 0.1 M PBS, and incubated in 0.3%–0.4% Triton X-100 for 2 h at room temperature. Sections were then rinsed with 0.1 M PBS, and incubated in Vectastain ABC Elite kit (Vector Laboratories, Burlingame, CA) solution for 3 h at room temperature. Following three PBS washes, sections were reacted with diaminobenzidine hydrochloride (Sigma, St. Louis, MO, USA) and nickel chloride (Merchenthaler et al. 1989). After BDA staining, a series of sections were counterstained with either cresyl violet or neutral red for Nissl substance. In some cases of V1, a series of sections were stained with cytochrome oxidase histochemistry (Wong-Riley 1979). Sections were mounted on gelatinized slides, air-dried, dehydrated, cleared, and coverslipped.

Data Analysis

Sections were examined with a light microscope (Eclipse E-800; Nikon, Tokyo, Japan) under bright-field illumination. Quantitative analysis was limited to cases in which the injection was successfully restricted to layers 2 and 3. We determined the laminar

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**Table 1** List of animals, injection location, and sizes

<table>
<thead>
<tr>
<th>TE</th>
<th>V1</th>
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<tr>
<td><strong>Age</strong></td>
<td><strong>Body wt (kg)</strong></td>
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<tr>
<td>PD 7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.45</td>
</tr>
<tr>
<td>PD 14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.64</td>
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<tr>
<td>PD 1</td>
<td>0.56</td>
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<tr>
<td>PD 7</td>
<td>0.77</td>
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<tr>
<td>PD 1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.51</td>
</tr>
<tr>
<td>PD 37</td>
<td>0.75</td>
</tr>
<tr>
<td>PD 65&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.98</td>
</tr>
<tr>
<td>PD 70</td>
<td>0.64</td>
</tr>
<tr>
<td>PD 71</td>
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<tr>
<td>PD 71</td>
<td>1.0</td>
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<tr>
<td>PM 7</td>
<td>1.6</td>
</tr>
<tr>
<td>PM 8&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>PM 10</td>
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<tr>
<td>Adult</td>
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<tr>
<td>Adult&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>Adult</td>
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<tr>
<td>Adult</td>
<td>10.4</td>
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<tr>
<td>Adult</td>
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<td>Adult</td>
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<tr>
<td>Adult</td>
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<tr>
<td>PD 7&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>PD 14&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>PD 27</td>
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<td>PD 27&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>PD 37</td>
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<td>PD 65&lt;sup&gt;b&lt;/sup&gt;</td>
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<td>PD 71</td>
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<sup>a</sup>Perfusion date as animal age.
<sup>b</sup>Injection cases which were selected for counting of boutons.
<sup>c</sup>The symbol “~” means “not determined”.

$sulcus$ were possibly located in TEm of Seltzer and Pandya (1978) or in CITd and AITd of Felleman and Van Essen (1991). In V1, the injections were made into the operculum representing visual fields of ~5° eccentricity (Daniel and Whitteridge 1961; Van Essen et al. 1984; Tootell et al. 1988).

**Histology**

Most animals were perfused 7 days after injection. Some adult animals survived 10–28 days. A 4-day-old infant survived 3 days after injection. The day of sacrifice was taken as the age of the monkeys (Table 1). Following anesthesia with pentobarbital overdose, heparin was injected into the heart after opening the chest cavity. The animals were then transcardially perfused with 0.5–1 L of phosphate-buffered saline (PBS, 37°C). Infants and juvenile monkeys, as well as a few adults, were fixed with 1–2 L of ice-cold 4% paraformaldehyde, 0.2% picric acid, 0.1% glutaraldehyde in 0.1 M PB followed by 0.8 L of ice-cold 4% paraformaldehyde in PBS. The contralateral hemispheres of these monkeys without injections were used for immunocytochemical studies (Xu et al. 2003). The other adult monkeys were fixed with 2 L of 4% ice-cold paraformaldehyde. BDA labeling quality did not differ between the animals fixed through the different fixative methods.

The brains were removed and photographed, then blocked, postfixed overnight in fixative, and soaked in 10–30% sucrose PBS for cryoprotection. The surface of the blocks of TE and V1 containing the injection sites was first flattened by pressing it against aluminum foil-covered dry ice, and then the whole blocks were frozen with dry ice powder. No physical unfolding of tissues was performed. Serial sections were cut tangentially to the flattened surface on a freezing microtome at 50 µm. Sections were rinsed with 0.1 M PBS, and incubated in 0.3–0.4% Triton X-100 for 2 h at room temperature. Sections were then rinsed with 0.1 M PBS, and incubated in Vectastain ABC Elite kit (Vector Laboratories, Burlingame, CA) solution for 3 h at room temperature. Following three PBS washes, sections were reacted with diaminobenzidine hydrochloride (Sigma, St. Louis, MO, USA) and nickel chloride (Merchenthaler et al. 1989). After BDA staining, a series of sections were counterstained with either cresyl violet or neutral red for Nissl substance. In some cases of V1, a series of sections were processed for cytochrome oxidase histochemistry (Wong-Riley 1979). Sections were mounted on gelatinized slides, air-dried, dehydrated, cleared, and coverslipped.
location of injection by examining sections containing the injection site and its surrounding region, stained for either cytochrome oxidase or Nissl substance (Supplementary Fig. 1). Patches were identified as clusters of labeled terminal ramifications spanning consecutive BDA-reacted sections. It was often difficult to estimate the size of the injection site, or the effective tracer-uptake zone, due to extensive labeling of horizontal and local recurrent axons and tracer residue around the injection site. To estimate the size of the effective tracer-uptake zone in layers 2 and 3, we measured the area containing labeled descending axon bundles in deeper sections; these labeled axon bundles should have originated from neurons successfully labeled at the tracer-uptake zone in layers 2 and 3 (Fujita and Fujita 1996). Because descending axon bundles are more compactly distributed in deeper layers due to the curvature of the cortex, we corrected the area containing labeled bundles in deeper sections using radially penetrating blood vessels as a reference. Specifically, we measured the distances between radially penetrating blood vessels in the upper section containing the injection site core and those in the deeper section containing descending axon bundles, and then calculated the expansion ratio of these distances (distances in upper section/distances in deeper sections). We then used this expansion ratio to correct the size of area containing labeled bundles (Tanigawa et al. 2005).

Photomicrographs of selected sections were captured under a microscope (×10) using a 3CCD color video camera (DXC-950; Sony, Tokyo, Japan). Montages were made using an image analysis system (MCID, Imaging Research, Inc., ON, Canada). Before processing for delineation of terminal patches, retrogradely labeled cells and blood vessels were filtered out from images. Terminal patches of horizontal axons were then delineated by applying an edge-detecting algorithm that uses the difference of 2 Gaussian filters (radii; 75 and 120 µm) to detect local maximum gradients of luminance intensity over the image (Marr 1982; Malach et al. 1993; for details, see Tanigawa et al. 2005). After patches were delineated, we determined the area, lateral extent, x–y coordinates relative to the injection center, and optical density of each patch by ImageJ (a public domain software developed at NIH).

Because the surface of the tissue containing the injection site was flattened before sectioning, the section plane was almost parallel to the surface around the injection site. However, far from the injection site, particularly near or within the sulci, the section was not cut parallel to the surface, potentially resulting in distortion of patch shape. Therefore, patches near the edges of sections were excluded from the size analysis. In addition, we may have missed some labeled patches in the banks of the sulci. This issue should be taken into account in interpreting the results of the number, maximal lateral extent, and distribution pattern of labeled patches; in particular, the former 2 measures might have been underestimated. The effect on the distribution pattern will be discussed in the Results section.

To obtain the labeling intensity of patches, we first subtracted the mean optical density of section background from those of individual labeled patches, and normalized them to that of the most densely labeled patch in the section. To measure the center-to-center distance between neighboring patches, it is necessary to define which patches are neighbors. Previous studies did not define this rigorously, and reported results are often difficult to interpret. We adopted the definition of Gabriel and Sokal (1969) (Gabriel graph): 2 patches whose centers are A and B are defined to be neighbors if all other centers of patches are outside the A–B circle in which A and B are at opposite points of the circumference. We measured interpatch distances between neighboring patches conforming to this definition.

To count the number of synaptic boutons, we placed a counting window (100 × 100 µm²) at the most densely stained region of individual patches. When we found retrogradely labeled cells in that region, we moved the window so as to avoid them. The total number of boutons within this area was counted using an ×100 oil-immersion objective. We measured interbouton intervals using a computer-aided plotting system (Neurolucida, MicroBrightField, Inc., Colchester, Vermont). For bouton counting, we did not use the dissector-based method (Sterio 1984). Although our counting method might have overestimated the number of boutons due to excessive counts on both surfaces of physical or optical sections (Mayhew and Gundersen 1996), we do not think that it critically affects our conclusion for the following 2 reasons (von Bartheld 2002). First, synaptic boutons are relatively small structures (~1 µm), therefore the overestimation of bouton counts on section surfaces would have been small compared with that of cell counts. Second, our conclusion relied on relative comparisons among the age groups or between TE and V1, for which we counted boutons using the same method. Biases in bouton numbers by our counting methods, if any, should occur in a similar way across the materials, maintaining relative relationships.

To estimate the developmental enlargement of TE and V1 cortices where we made injections, we used gyral and sulcal landmarks around our injection sites. We measured the distance along the anteroposterior axis from the anterior end of lunate sulcus to the posterior pole of hemisphere for V1 and the distance from the anterior end of superior temporal sulcus to the anterior end of posterior middle temporal sulcus for TE. These distances were measured immediately after perfusion. In V1, we found no significant difference in the linear distance among the 4 age groups; postnatal days 7–27 (PD 7–27), 37–71 (PD 37–71), postnatal months 7–10 (PM 7–10), and older than 3 years (adults) (Kruskal–Wallis test, P = 0.14). In contrast, in TE, the linear distance was significantly different among the 4 age groups (Kruskal–Wallis test, P = 0.01). The distance in adults was increased by 19% from PD 7–27, by 10% from PD 37–71 and PM 7–10. For all measurements, no corrections were made for tissue shrinkage induced by tissue perfusion and processing.

Results

First, we describe “qualitatively” the morphological features of patches of horizontal axons in TE and V1 at different postnatal stages. Second, we analyze these features “quantitatively,” confirming the differences in patch topography between TE and V1 during all phases of postnatal development. Third, we examine the finer morphology of axons within patches. Finally, we demonstrate that the density of synaptic boutons within patches underwent area-specific developmental changes in TE and V1.

Qualitative Description of Terminal Patches of Labeled Horizontal Axons

In a 1-week-old monkey, we found distinct patches of horizontal axon ramifications originating from the injections (Figs 1 and 2). At first glance, these labeled patches were indistinguishable from those observed in adult animals. Morphological differences of patches between TE and V1 were evident at this early stage and remained appreciable in all ages examined. TE patches appeared to be larger, more loosely packed, irregularly spaced, and more widely distributed in an extensive cortical region than those in V1. Some injections in TE produced patches were distributed primarily along preferred directions (Fig. 1A, B, C–L). Other injections
labeled patches without a significant bias in distribution (Fig. 1C–F). In V1, the distribution of patches in most cases assumed an oval appearance, elongated along a particular axis (Fig. 2A–F,I–L). Other injections produced a patch distribution with a more circular, "rosette"-like pattern (Fig. 2G,H), as reported for adult V1 (Amir et al. 1993). Infant and juvenile monkeys had more patches in V1 than adults (Fig. 2). Note that even in an extensively labeled case (Fig. 2C,D), the injection core and labeled patches were observed in the section at the depth of 450–500 µm, which correspond approximately to deep layer 3 (Blasdel et al. 1985).

The pattern of labeled patches was consistent across serial sections throughout layers 2 and 3 (Supplementary Fig. 2); therefore, observed patch patterns were not fortuitous results in selected sections. Differences in patch morphology between TE and V1 did not result from differences in the degree of tissue fixation and shrinkage; the samples of TE and V1 injection cases in Figures 1 and 2 derived from the same animals at corresponding ages, with the exception of the adult monkeys.

**Quantitative Morphological Analyses of Patches**

We quantitatively analyzed the morphological features of patches, such as the size, spacing, spread, distribution and labeling intensity pattern, in TE and V1 to evaluate the differences between these 2 cortical areas and the changes in these variables during postnatal development. Animals were divided into 4 age groups based on the stage of visual system development: postnatal days 7–27 (PD 7–27), 37–71 (PD 37–71), postnatal months 7–10 (PM 7–10), and older than 3 years (adults). The first period, PD 7–27, is before the peak in postnatal synaptogenesis (Rakic et al. 1986) and spinogenesis in layer III pyramidal cells (Elston et al. 2009, 2010). The second period corresponds to the peak period of synaptogenesis and spinogenesis. Synaptogenesis in the cerebral cortex is maintained in a plateau phase during the third period (Rakic et al. 1986). The fourth group consists of mature adults in which both synaptogenesis and spinogenesis have decreased to their adult levels. Data were sampled from 8, 7, 7, and 5 animals in PD 7–27, PD 37–71, PM 7–10, and adults, respectively (Table 1).

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**Figure 1.** Terminal patches of horizontal axons in TE at different postnatal ages. The left panels show photomicrographs of representative tangential sections of BDA-injected TE. The right panels show reconstructions of the injection sites and labeled patches shown in the left panels. Varying shading in the reconstructions indicates the optical density of patch labeling normalized to the most densely stained patch in each section. The striped area indicates the "halo" of the injection site; the filled circle inside the halo indicates the injection site. The reconstructions are shown in a view from above the cortical surface. The lateral views of the brain (insets in the right panels) show the injection sites (dots) and areas where the reconstructions were performed (shaded areas). STS, superior temporal sulcus. Scale bars, 1 mm.
The size of tracer injections affects the size, number, and lateral extent of labeled terminal patches of horizontal axons (Lund et al. 1993; Malach et al. 1997; Tanigawa et al. 2005). We, therefore, compared injection sizes among different age groups (Table 1). The injection size did not differ among the 4 age groups in V1 (0.05–0.10 mm²; Kruskal–Wallis test, $P = 0.43$) and among the 3 age groups (0.05–0.10 mm²; $P = 0.057$) in TE, with the exception of adults (0.14 ± 0.18 mm²). The injection size did not differ between TE and V1 in any age group (Mann–Whitney U-test, $P > 0.05$ after Bonferroni correction for multiple comparisons), with the exception of adults ($P < 0.001$). Although injections in adult TE were larger than in the other groups, the injection size correlated with neither the patch number (Spearman rank correlation test, $P = 0.15$ for TE, $P = 0.89$ for V1) nor the maximal lateral extent of patch distribution ($P = 0.22$ for TE, $P = 0.48$ for V1). This indicates that variations in injection size was modest in the present study and did not systematically affect the parameters of labeled patches.

Another technical issue is that BDA, the anterograde tracer we used, occasionally labels neurons retrogradely, as previously reported (Shmuel et al. 2005). In our sections of deep layer 3, we also found a small number of retrogradely labeled neurons inside axonal patches. On average, 0.2–4.8 retrogradely labeled neurons were present in individual patches within a single section. These labeled neurons could result in secondary axonal labeling, leading to overestimation of the maximum lateral extent of patches. For each injection case, we counted the number of labeled neurons in patches across 3 consecutive sections (total thickness, 150 µm) in deep layer 3. We found no significant difference among age groups in either TE or V1 (Kruskal–Wallis test, $P = 0.41$ for TE, $P = 0.1$ for V1). If the number of retrogradely labeled neurons correlated with the maximum lateral extent, injection cases in which a larger number of neurons were labeled should have had a greater lateral extent of patches; however, this was not the case. The 2 injection cases of TE and V1 in infant monkeys (PD 37 and PD 65) yielded the smallest number of retrogradely labeled neurons, yet the maximum lateral extent of labeled axon patches was greater than in any adult cases. We often observed horizontal axons passing through patches on the way to more distant patches, but did not find horizontal axons arising from retrogradely labeled neurons that were only faintly labeled. Taken together, these observations indicate that morphological measurements of patches were unlikely to be affected by retrograde labeling to an extent that would change our conclusions.

**Number of Labeled Patches**

In TE, the average number of patches projecting from individual injections was 17, 20, 21, and 19 (Fig. 3A; SD = 4, 4, 5, and 9) in PD 7–27, PD 37–71, PM 7–10, and adults, respectively, and did not...
differ among the 4 age groups (Kruskal–Wallis test, $P = 0.26$). In V1, the average patch number was 32, 30, 16, and 12 ($SD = 3, 7, 6$, and 5), respectively. The number of patches in V1 differed among the 4 age groups (Kruskal–Wallis test, $P = 0.001$), decreasing with age. Pairwise comparison of TE and V1 within each age group demonstrated that patch numbers were larger in V1 than in TE in PD 7–27, but not in the other age groups (Mann–Whitney U-test, $P < 0.05$ after Bonferroni correction).

**Maximal Lateral Extent of Patch Distribution**

The average maximal lateral extent in TE was 6.15, 7.16, 6.63, and 5.62 mm (Fig. 3B; $SD = 1.24, 1.32, 1.32$, and 1.76 mm) in PD 7–27, PD 37–71, PM 7–10, and adults, respectively. Statistical analyses revealed no significant difference among the 4 age groups (Kruskal–Wallis test, $P = 0.32$). In V1, however, the average maximal lateral extent was 2.83, 2.49, 1.89, and 1.34 mm ($SD = 0.48, 0.62, 0.69$, and 0.38 mm), respectively, and a significant difference was observed among the 4 age groups ($P < 0.001$). The maximum lateral extent thus decreased with age in V1. In all age groups, the maximal lateral extent of patch distribution was 2.0–5.4 times greater in TE than in V1 (Mann–Whitney U-test, $P < 0.05$ after Bonferroni correction).

The description here applies only to patches delineated according to the method described in Materials and Methods. In V1, axons that did not form clear patches could be traced beyond the farthest patches. The average maximum horizontal projection of labeled axons was 4.14, 4.22, 4.24, and 3.67 mm ($SD = 0.82, 1.58, 0.68$, and 0.53 mm) in PD 7–27, PD 37–71, PM 7–10, and adults, respectively, and no significant difference was observed among the 4 age groups (Kruskal–Wallis test: $P = 0.69$).

**Interpatch Distance**

In TE, the average interpatch distance, defined as the center-to-center distance between 2 neighboring patches, was 1.50, 1.43, 1.28, and 1.20 mm (Fig. 3C; $SD = 0.69, 0.65, 0.72$, and 0.65 mm) in PD 7–27, PD 37–71, PM 7–10, and adults, respectively, thus gradually decreasing with age (Kruskal–Wallis test, $P < 0.001$; see Materials and Methods for definition of “neighboring patches”). In V1, the average interpatch distance was 0.64, 0.63, 0.65, and 0.63 mm ($SD = 0.19, 0.16, 0.22$, and 0.22 mm), respectively, and

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**Figure 2.** Terminal patches of horizontal axons in V1 at different postnatal ages. Conventions are the same as in Figure 1. The drawings of occipital operculum (insets in the right panels) are viewed from above the cortical surface. Except for adult monkeys, the same animals provided sections for V1 in this figure and for TE in Figure 1, at corresponding ages. IOS, inferior occipital sulcus; LS, lunate sulcus; D, dorsal; M, medial. Scale bars, 1 mm.
no significant difference was observed among the 4 age groups (Kruskal–Wallis test, $P = 0.58$). Throughout postnatal development, the interpatch distance was 1.9–2.3 times greater in TE than in V1 (Mann–Whitney $U$-test, $P < 0.005$ after Bonferroni correction).

We assessed the variation of interpatch distances by calculating the ratio of the standard deviation to the mean (coefficient of variance, CV). In TE, the average CV was 0.46, 0.44, 0.56, and 0.44 (SD = 0.07, 0.06, 0.09, and 0.10) in PD 7–27, PD 37–71, PM 7–10, and adults, respectively. In V1, the average CV was 0.28, 0.25, 0.26, and 0.22 (SD = 0.04, 0.02, 0.10, and 0.05), respectively. We found no significant difference in CV among the 4 age groups in either TE or V1 (Kruskal–Wallis test, $P = 0.09$ for TE, $P = 0.1$ for V1). CV was larger in TE than in V1 for all age groups (Mann–Whitney $U$-test, $P < 0.05$ after Bonferroni correction). Thus, TE exhibited a more irregular spacing of patches than V1 throughout postnatal development.

**Size of Patches**

In TE, the average patch size was 0.178, 0.184, 0.202, and 0.178 mm$^2$ (Fig. 3D; SD = 0.090, 0.100, 0.113, and 0.097 mm$^2$) in PD 7–27, PD 37–71, PM 7–10, and adults, respectively. The average patch size in V1 was 0.085, 0.089, 0.095, and 0.087 mm$^2$ (SD = 0.029, 0.034, 0.039, and 0.044 mm$^2$), respectively. In either TE or V1, no change was observed in the patch size among the 4 age groups (Kruskal–Wallis test, $P = 0.39$ for TE, $P = 0.32$ for V1). The size of patches was similar to the size reported previously in adult monkeys (TE: 0.2 ± 0.08 mm$^2$, Fujita and Fujita 1996; V1: 0.068 mm$^2$, Amir et al. 1993). Throughout postnatal development, the size of patches was 1.9–2.4 times greater in TE than in V1 (Mann–Whitney $U$-test, $P < 0.005$ after Bonferroni correction).

**Distribution Pattern of Patches**

As a measure of patch distribution around the injection site, we calculated an anisotropy ratio (Malach et al. 1993; Fujita and Fujita 1996; Yoshioka et al. 1996; Tanigawa et al. 2005). For this index, we drew a rectangle that included all patch centers and maximized the length:width ratio of the rectangle. A higher value of this index indicates that patches tend to spread more along a certain axis. In V1, the average anisotropy ratio was 2.07, 1.45, 1.74, and 2.06 (Fig. 3E; SD = 0.89, 0.32, 0.72, and 0.78) in PD 7–27, PD 37–71, PM 7–10, and adults, respectively, and did not differ among the 4 age groups (Kruskal–Wallis test, $P = 0.53$); the degree of elongation of patch distribution in V1 was similar throughout postnatal development.
To further examine the developmental changes in the distribution pattern of patches, we calculated another measure of patch distribution, the asymmetry ratio (Malach et al. 1997). To calculate this ratio, we first drew an imaginary line through an injection site, bisecting the cluster of patches to give the most unbalanced distribution. We then counted the number of patches on either side of this bisector, and obtained the ratio by dividing the larger number by the smaller one. The asymmetry ratio reflects the tendency of patches to be distributed more frequently on one side of the injection site. In V1, the average asymmetry ratio was 1.32, 1.41, 1.38, and 1.53 (Fig. 3F; SD = 0.15, 0.39, 0.33, and 0.38), in PD 7–27, PD 37–71, postnatal months (PM) 7–10, and adults, respectively, and did not differ among the 4 age groups (Kruskal–Wallis test, P = 0.74). Our analyses of anisotropy and asymmetry ratios suggest that an adult-like distribution pattern of patches in V1 forms during early life.

We also calculated these 2 indices for patches in TE. The average anisotropy ratio was 1.86, 2.29, 2.37, and 2.26 (Fig. 3E; SD = 0.31, 0.73, 0.41, and 0.67) in PD 7–27, PD 37–71, PM 7–10, and adults, respectively, and the average asymmetry ratio was 4.73, 2.89, 2.89, and 4.64 (Fig. 3F; SD = 2.93, 1.24, 1.33, and 2.33), respectively. In TE, neither the anisotropy nor asymmetry ratio differed among the 4 age groups (Kruskal–Wallis test, P = 0.25 and P = 0.24). We found no significant difference in the anisotropy ratio between TE and V1 in any age group (Mann–Whitney U-test, P > 0.05 after Bonferroni correction), whereas the asymmetry ratio was greater in TE than in V1 for all age groups (Mann–Whitney U-test: P < 0.05 after Bonferroni correction).

In TE, these 2 indices must be interpreted with caution. Because we did not unfold the sulci before sectioning (see Materials and Methods), we might have missed some labeled patches in the banks of superior temporal sulcus and anterior middle temporal sulcus in the analysis. Thus, the indices we measured might reflect the distribution of patches in the gyral part of TE, but not the overall distribution of patches across the gyral and sulcal parts of TE. We addressed this issue by analyzing the labeled patches in one particular injection case (PD 65) for which we kept sections up to a depth of 3.5 mm below the gyral surface into the superior temporal and anterior middle temporal sulci. In the initial analysis, the patch distribution was elongated anteroposteriorly. We encountered some new patches that had been missed in the initial analysis (Fig. 4), located along the axis of patch distribution. Even taking patches in the banks of sulci into account, the distribution of patches remained anisotropic in this case.

**The Relationship Between Labeling Intensity of Patches and Projection Distance**

To estimate the connection density between the injection site and its projection targets, we measured the optical density of labeled patches. This analysis served to detect developmental changes in the pattern of connection density, but not the absolute density of labeling. We selected a representative section from each injection case, normalizing the optical density of individual patches to that of the densest patch, to obtain their relative labeling intensity (see Materials and Methods).
In V1, all injections cases demonstrated a negative correlation between the relative labeling intensity of patches and their projection distance (Fig. 5 right; Spearman’s correlation rank test; P < 0.05), except in one adult (P = 0.06), where only 6 patches were labeled just around the injection site (<1 mm away from the injection center). In the following analysis of this section, we excluded injection cases with the maximal lateral extent of <1 mm from the analysis (one case of PM 7–10 and 3 cases of Adults in V1). The average Spearman’s correlation coefficient ($r_s$) was −0.92, −0.92, −0.90, and −0.87 (SD = 0.05, 0.05, 0.12, and 0.09) in PD 7–27, PD 37–71, PM 7–10, and adults, respectively. No differences were observed among the 4 age groups (Kruskal–Wallis test, P = 0.43). In TE, 70% of injections demonstrated a significant decline of labeling intensity with projection distance (Fig. 5 left; Spearman’s correlation rank test; P < 0.05); 30% showed no significant correlation. The average $r_s$ was −0.44, −0.51, −0.68, and −0.67 (SD = 0.40, 0.27, 0.12, and 0.18), respectively, without a significant difference among the 4 age groups (Kruskal–Wallis test, P = 0.46). $r_s$ was greater in V1 than in TE in all age groups (Mann–Whitney U-test, P < 0.05 after Bonferroni correction). The connection density thus steeply declines for farther patches in V1, whereas this relation is weaker or even absent for TE.

The density of inter-areal and intrinsic connections probed by retrograde tracers decays exponentially with projection distance (exponential distance rule; Markov et al. 2011; Ercsey-Ravasz et al. 2013). Here, we examined whether the patch labeling intensity followed this rule in each age group. Using the least-squares method, we fitted exponential regression curves to the plots of patch-labeling intensity against projection distance (Fig. 5). The formula for the curve is

$$y = c \exp(-\lambda x),$$

where $x$ is the projection distance (mm), $y$ is the patch-labeling intensity, and $c$ is a normalization constant. Another constant, $\lambda$, represents the decay rate of connection density with projection distance; specifically, connection density is reduced to $1/\exp(\lambda)$ per mm. To estimate the goodness of fit of the regression curves, we calculated the determination coefficient ($R^2$). In V1, the average $R^2$ of best-fit exponential regression curves was 0.84, 0.82, 0.75, and 0.84 (SD = 0.07, 0.02, 0.19, and 0.12) in PD 7–27, PD 37–71, PM 7–10, and adults, respectively. There was no significant difference among the 4 age groups (Kruskal–Wallis test, $P = 0.73$). In TE, the average $R^2$ of exponential regression curves was 0.33, 0.33, 0.37, and 0.44 (SD = 0.26, 0.28, 0.14, and 0.26), respectively, and did not differ significantly among the 4 age groups (Kruskal–Wallis test, $P = 0.66$). $R^2$ was greater in V1 than in TE in all age groups (Mann–Whitney U-test, $P < 0.05$ after Bonferroni correction), indicating that the exponential distance rule holds more precisely in V1 than in TE throughout postnatal development.

We next investigated whether the data were fitted better by an exponential regression than by a simple linear regression. In V1, the average $R^2$ of linear regression lines was 0.67, 0.67, 0.73, and 0.72 (SD = 0.11, 0.07, 0.15, and 0.08) in PD 7–27, PD 37–71, PM 7–10, and adults, respectively, whereas in TE, the average $R^2$ of linear regression lines was 0.30, 0.29, 0.39, and 0.38 (SD = 0.24, 0.25, 0.12, and 0.25), respectively. In each age group, and in both TE and V1, there was no significant difference in $R^2$ between these 2 types of regression (Wilcoxon signed-ranks rest, $P > 0.05$ after Bonferroni correction). However, when the data from all age groups were combined, $R^2$ was greater for the exponential regression than for the linear regression in both TE and V1 (Wilcoxon signed-ranks rest, $P = 0.00004$ for V1; $P = 0.02$ for TE). Thus, the decay of connection density with projection distance was better described by an exponential function than by a linear function both in TE and V1.

In V1, the average value of $\lambda$ was 1.52, 1.76, 1.55, and 2.97 mm$^{-1}$ (SD = 0.39, 0.47, 0.33, and 0.80 mm$^{-1}$) in PD 7–27, PD 37–71, PM 7–10, and adults, respectively, and did not differ significantly among the 4 age groups (Kruskal–Wallis test, $P = 0.01$). In particular, a significant difference was observed between PD 7–27 and adults (Mann–Whitney U-test, $P < 0.05$ after Bonferroni correction), suggesting that the connection density of horizontal axons in V1 declines more sharply in adults than in infants. In TE, the average value of $\lambda$ was 0.23, 0.34, 0.28, and 0.43 mm$^{-1}$ (SD = 0.29, 0.30, 0.13, and 0.24 mm$^{-1}$), respectively, and did not differ significantly among the 4 age groups (Kruskal–Wallis test, $P = 0.29$). $\lambda$ was greater in V1 than in TE in all age groups (Mann–Whitney U-test, $P < 0.05$ after Bonferroni correction). The analysis of $\lambda$ confirmed that the exponential decay of connection density with projection distance is sharper in V1 than in TE throughout postnatal development.

**Morphology of Axonal Arbors Within Patches**

In contrast to rather modest, yet consistent, changes in the morphology of terminal patches throughout postnatal development, horizontal axon arbors within individual patches underwent striking changes. Higher-magnification photomicrographs of patches in monkeys of 4 different ages (Fig. 6) demonstrated that horizontal axonal arbors in both TE and V1 were straighter, possessing a smaller number of varicosities at PD 7 (Fig. 6A,B), than in adults (Fig. 6C,H). Axonal arbors in PM 2–8 demonstrated an intermediate morphology between PD 7 and adults (Fig. 6C–F).

Horizontal axon terminals frequently accompanied growth cones at their tips in TE and V1 of 1-week-old monkeys (arrows in Fig. 6A,B). Many growth cones possessed spike-like protrusions (insets in Fig. 6A,B). Some growth cones had only a central core, lacking protrusions (right inset in Fig. 6A). In 2- and 3-week-old monkeys, the number of growth cones decreased dramatically, rarely possessing spike-like protrusions. In monkeys older than 4 weeks, we seldom encountered growth cones (Fig. 6C–H).
Both in TE and V1, horizontal axons at PD 7 bore mostly en passant boutons (swellings along an axonal branch, Fig. 7A,B); those in adults bore both en passant boutons and terminaux boutons (swellings at the end of a short connecting stalk, Fig. 7C,D). Qualitative observation of horizontal axons showed that main trunks of axons outside patches bore fewer boutons in both TE and V1 at PD 7 (Fig. 7E,F) as well as in adults (Fig. 7G,H), as compared with those inside patches. These results are consistent with previous report by Gilbert and Wiesel (1983), who made a reconstruction of horizontal axons of pyramidal neurons in layer 3 by intracellular injection and showed more boutons inside patches than outside patches.

Synaptic Boutons Within Patches

Synaptic boutons, both en passant and terminaux, are sites of synaptic contact (McGuire et al. 1991; Melchitzky et al. 1998). To determine whether changes in the complexity of axonal arbors within patches accompanied changes in bouton density, we counted both types of boutons in TE and V1 in selected injection cases (Table 1). For each age group, we selected the same number of injection cases for TE and V1 (n = 3, 1, 1, and 2 injection cases in PD 7–27, PD 37–71, PM 7–10, and adults, respectively). We examined only patches with the same range of projection distances across the 4 age groups (within 3.0 mm for TE and 1.5 mm for V1). We examined 33, 5, 15, and 45 patches, respectively, in TE and 65, 18, 15, and 20 patches, respectively, in V1. We counted boutons at the most densely labeled regions of individual patches (see Materials and Methods).

In TE, the average number of boutons per 100 × 100 μm² area was 68.4, 140.2, 107.5, and 171.9 (Fig. 8; SD = 25.9, 26.8, 30.7, and 96.7) in PD 7–27, PD 37–71, PM 7–10, and adults, respectively. In V1, the average number of boutons was 92.8, 111.3, 164.5, and 176.1 (SD = 55.5, 52.9, 73.8, and 58.4), respectively. In both TE
and V1, the density of boutons differed among the 4 age groups (Kruskal–Wallis test, \( P < 0.001 \) for both TE and V1), increasing from infancy to adulthood. Bouton density was similar between TE and V1 in all age groups (Mann–Whitney U-test, \( P > 0.05 \) after Bonferroni correction).

Changes in the bouton density within patches are likely to result, in part, from increases in branching of horizontal axon terminals (Fig. 6). We could not, however, obtain quantitative data on the branch number due to difficulties reconstructing single axons from densely labeled patches. Changes in the bouton density could also accompany shortening of interbouton intervals because of new bouton formation on existing axons. In TE, the median of interbouton intervals was 11.8, 11.2, 9.2, and 8.2 µm in PD 7–27, PD 37–71, PM 7–10, and adults, respectively (Fig. 9A). The median in V1 was 10.4, 9.4, 8.7, and 8.6 µm, respectively (Fig. 9B). In both TE and V1, the interbouton interval differed among the 4 age groups (Kruskal–Wallis test, \( P < 0.001 \) for both TE and V1) and decreased with age. The results suggest that newboutons are formed on existing axons during postnatal development and contribute to the increase in bouton density from infancy.

The Relationship Between Bouton Density Within Patches and Projection Distance
In V1, all injection cases demonstrated a negative correlation between the density of boutons and projection distance (Fig. 10).
right; Spearman rank correlation test, \( P < 0.001 \), indicating that decreases in bouton density with projection distance were consistently observed in all age groups. The average Spearman’s correlation coefficient \( (r_s) \) between the bouton density and projection distance was \(-0.85, -0.82, -0.78, \) and \(-0.84 \) (SD = \( 0.06, N/A, N/A, \) and \( 0.14 \)) in PD 7–27, PD 65, PM 8, and adults, respectively. In TE, a negative correlation was also observed in 5 of 7 selected injection cases (Fig. 10 Left; Spearman rank correlation test, \( P < 0.05 \)). The average \( r_s \) was \(-0.72, -0.5, -0.69, \) and \(-0.44 \) (SD = \( 0.16, N/A, N/A, \) and \( 0.44 \)), respectively. When the data from all age groups were combined, \( r_s \) was greater in V1 than in TE (Mann–Whitney \( U \)-test, \( P = 0.03 \)). This result is consistent with our finding revealed by the analysis of labeling intensity of patches.

We also investigated whether the exponential distance rule holds for the relationship between the bouton density and projection distance. To this end, we fitted exponential regression curves, as in the analysis of patch labeling intensity, to the plots of selected injection cases using the least-squares method. The fitting curves were defined by formula 1, with \( y \) in this case representing the density of boutons. The average \( R^2 \) of best-fit exponential regression curves was \( 0.79, 0.74, 0.69, \) and \( 0.71 \) (SD = \( 0.07, N/A, N/A, \) and \( 0.03 \)) in V1, and \( 0.57, 0.26, 0.49, \) and \( 0.27 \) (SD = \( 0.22, N/A, N/A, \) and \( 0.32 \)) in TE, in PD 7–27, PD 65, PM 8, and adults, respectively. When the data from all age groups were combined, \( R^2 \) was greater in V1 than in TE (Mann–Whitney \( U \)-test, \( P = 0.002 \)). This is consistent with the results obtained from our analysis of the labeling intensity (Fig. 5). For comparison, we also fitted linear regression lines to the plots. The average \( R^2 \) of linear regression lines was \( 0.71, 0.70, 0.69, \) and \( 0.71 \) (SD = \( 0.11, N/A, N/A, \) and \( 0.03 \)), respectively, in V1, and \( 0.57, 0.26, 0.45, \) and \( 0.27 \) (SD = \( 0.22, N/A, N/A, \) and \( 0.27 \)), respectively, in TE. In contrast to the results of the analysis of labeling intensity, there was no significant difference in \( R^2 \) between these 2 types of regression, even though the data from all age groups were combined (Wilcoxon signed-ranks test, \( P = 0.22 \) for V1; \( P = 0.47 \) for TE). This might be because the data for bouton density was limited to a small number of cases. Another possible explanation is that bouton density may be more variable and depend on the location where it is counted. The average value of \( \lambda \) was \( 0.97, 1.21, 1.19, \) and \( 1.20 \) mm \(^{-1} \) (SD = \( 0.45, N/A, N/A, \) and \( 0.02 \) mm \(^{-1} \)), respectively, in V1, and \( 0.28, 0.07, 0.15, \) and \( 0.20 \) mm \(^{-1} \) (SD = \( 0.24, N/A, N/A, \) and \( 0.10 \) mm \(^{-1} \)), respectively, in TE. When the data from all age groups were combined, \( \lambda \) was greater in V1 than in TE (Mann–Whitney \( U \)-test, \( P = 0.001 \)), consistent with the results from the analysis of labeling intensity.

**Discussion**

In this study, we demonstrated that terminal patches of horizontal axons were present in both TE and V1 by PD 7. From this early stage of postnatal development up to adulthood, patches in TE were larger and more varied in size, more widely spaced, and more irregularly and extensively distributed than those in V1 (Fig. 11A, B). The connection density of terminal patches declined exponentially with projection distance in both areas, but in TE this trend was less evident (or even absent in some injection cases), at all ages examined. After birth, the terminal patches in these 2 areas followed divergent maturation profiles that shared some characteristics. Of particular note was the decrease in the lateral extent of patches in V1 from PD 7 to adulthood, whereas area TE maintained the lateral extent of patch distribution over the same period. In V1, connection density increased postnatally for patches close to injection sites, whereas in TE the connection...
became denser for a subset of patches located at various distances. Thus, our data reveal that axonal branches and boutons within patches continue to develop over many months, with connections being enhanced, or lost, in an area-specific manner in both areas (Fig. 11C,D). Below we discuss these findings in relation to results obtained in other species, as well as in macaque, and relate them to functional changes observed during development of visual cortex.

Formation of Area-specific Patches of Horizontal Axons

In the visual cortex of cats and ferrets, terminal patches of horizontal axons emerge postnatally from initial diffuse projections. The projection of horizontal axons in area 17 is diffuse during the early postnatal period, becoming patchy prior to eye opening at PD 8–10 in cats (Callaway and Katz 1990; Luhmann, Singer, et al. 1990; Lübke and Albus 1992; Galuske and Singer 1996) and at PD 27 in ferrets (Durack and Katz 1996; Ruthazer and Stryker 1996). This process involves the elimination of exuberant axons at inappropriate targets and the additional branching of horizontal axons at appropriate targets, rather than death of the cells of origin (Katz and Callaway 1992; Katz and Shatz 1996). Horizontal axons in V1 and V2 of macaque, however, attain this patchy pattern prior to birth. The projection of horizontal axons in layers 2 and 3 of V1 and V2 is diffuse by embryonic day 118 (ED 140), becoming patchy around ED 140–145, that is, 3 weeks before birth (Coogan and Van Essen 1996). Similarly, ipsilateral (Coogan and Van Essen 1996; Barone et al. 1996) and callosal (Meissirel et al. 1991; Schwartz and Goldman-Rakic 1991) corticocortical projections are initially diffuse, and then become patchy before birth. Although no study has investigated terminal patches of horizontal axons in prenatal TE, and further studies are obviously needed, the maturity of horizontal axons at PD 7 (injection at PD 4) suggests that a patchy projection pattern in TE is established in utero. Thus, patch formation does not likely require visual experience in either the striate cortex or extrastriate cortex in macaques.

The size, spacing, and distribution pattern of terminal patches differed markedly between TE and V1 at 1 week of age. In V1, the number and maximal lateral extent of patches decreased with age from PD 7 to adulthood. This decrease, without an accompanying change in interpatch distance, suggests that the outermost patches in V1 are eliminated postnatally (Fig. 11D), consistent with physiological studies in ferret and cat primary visual cortex (Luhmann, Greuel, et al. 1990; Nelson and Katz 1995). In contrast, the lateral extent of terminal patches did not change postnatally in TE (Fig. 11B). The bouton density in the patches increased from PD 7 to adulthood in both TE and V1. In TE, the increase in bouton density occurred in several patches at varying distances from the injection site. In V1, on the other hand, the increase was confined to patches close to the injection site. These increases in bouton density indicate that maturation of patches continues after birth, shaping their connection densities in an area-specific manner in TE and V1. Some of these developmental changes may reflect differences in the efficiency of tracer uptake and/or transport between younger animals and adults. This is unlikely especially with regard to the lateral extent, however, because the lateral extent did not change significantly across age groups in TE.

The factors that determine area-specific patch formation and refinement remain unknown; however, it is interesting to correlate the present data with developmental profiles of dendritic trees of layer 3 pyramidal cells, as the majority (> 90%) of presynaptic boutons of horizontal axons in terminal patches contact dendritic spines of these neurons (Melchitzky et al. 1998). Patch size in adult V1 is similar to that in basal dendritic trees of layer 3 pyramidal neurons, and the size of these structures increases concurrently with anterior progression along the ventral
and dorsal visual streams (Lund et al. 1993; Elston and Rosa 1997, 1998). Like the differences in horizontal axons, differences in the sizes of dendritic trees of layer 3 pyramidal cells in TE and V1 of the adult reflect differences in the development profiles of these 2 cortical areas (Elston et al. 2009, 2010). Specifically, in V1, the basal dendritic trees of layer 3 pyramidal cells decrease in size between early onset of visual experience and adulthood, whereas those in area TE continue to grow from birth to adulthood (Elston et al. 2010). Moreover, the dendritic trees of pyramidal cells in V1 lose more spines than they grow between the onset of visual experience and adulthood, whereas those in area TE grow more spines than they lose (Lund et al. 1977; Elston et al. 2010). Thus the developmental profiles of horizontal axon patches parallel those of dendritic trees of layer 3 pyramidal cells.

**Postnatal Refinement of Horizontal Connections in TE and V1**

It has been hypothesized that neural connections are maintained or strengthened between coactive presynaptic and postsynaptic neurons, and weakened or eliminated between neurons without correlated activities (Hebb 1949; LeVay 1988; Löwel and Singer 1992; Katz and Shatz 1996). The developmental change in patches of horizontal axons in V1 is consistent with this hypothesis. V1 is organized in a precise retinotopic manner (Hubel and Wiesel 1974), with horizontal axons linking cortical sites in which neurons share preferred stimulus selectivity (Gilbert and Wiesel 1989; Malach et al. 1993; Yoshioka et al. 1996; Bosking et al. 1997). V1 neurons in nearby sites with a similar stimulus preference will therefore be activated together more frequently than those in distant sites over the course of ongoing visual experience. This hypothesis predicts that the horizontal connections between nearby sites will strengthen, whereas those between distant sites will weaken, and this result is consistent with our findings.

In contrast, TE neurons have a large receptive field (>15–40°) that includes the fovea and overlaps with other neurons’ receptive fields. In addition, unlike V1, TE has no discernable retinotopic map (Gross et al. 1972; Desimone and Gross 1979; Fujita et al. 1992; Fize et al. 2003). Thus, TE neurons in nearby sites do not necessarily have a higher probability of co-activation on the basis of retinotopy. TE neurons in adults respond selectively to complex visual features; neurons with shared stimulus preferences are organized into multiple columns separated by a distance similar to the interpatch distance of horizontal axons (Fujita et al. 1992; Fujita and Fujita 1996; Wang et al. 1996, 2000). Although it remains unknown how TE neurons respond to visual stimuli immediately after birth, TE neurons in 6-week old monkeys already exhibit object-feature selectivity and large bilateral receptive fields (Rodman et al. 1991, 1993). Neurons with similar selectivity in infants are likely to be active together regardless of their cortical position in TE. Patches connected by horizontal axons may become stronger through visual experience if neurons within those patches respond to similar object features or to stimuli occurring together in natural scenes.

**Exponential Distance Rule in Intrinsic Horizontal Projections**

Markov et al. (2011) showed that the number of retrogradely labeled cells in macaque V1 decays exponentially with distance from the tracer injection site. In that study, a large population of counted cells were labeled through local short-range circuitry and included inhibitory neurons. In this study, we used labeling intensity or bouton density within axonal patches to estimate the connection density. Our results indicate that the exponential distance rule holds up even when we confine our analysis to long-range horizontal connections emanating from pyramidal neurons in V1. In TE, the average decay rate λ, calculated from patch-labeling intensity, ranged from 0.23 to 0.42 mm\(^{-1}\) across all age groups. These values were significantly smaller than those in V1 (from 1.52 to 2.97 mm\(^{-1}\)), and larger than λ estimated for the distance probability of inter-areal connections (0.188 mm\(^{-1}\); Ercey-Ravasz et al. 2013). Thus, the density of horizontal connections in TE decays more slowly than in V1 and more quickly than inter-areal connections.

The lower R\(^2\) (0.33–0.44) of exponential regression in TE throughout postnatal development indicates that factors other
than projection distance also play a role in determining the connection density of horizontal axons in TE. One possible explanation for this result is that some of distant labeled patches were formed by inter-areal projections. In the inferior temporal cortex, several different parcellation schemes have been proposed (e.g., Bonin and Bailey 1947; Seltzer and Pandya 1978; Yukie et al. 1990; Felleman and Van Essen 1991) but the area boundaries are still poorly defined at present. Axonal termination in layers 2 and 3 has been classified as that of lateral projections according to the model of Felleman and Van Essen (1991), but such projections are also found in inter-areal projections between neighboring areas such as V1–V2 and TEO–TE (Rockland and Virga 1989; Saleem et al. 1993; Anderson and Martin 2009). Another explanation may lie in the functional subdivisions of TE. In this area, there are several functional domains that span several millimeters across the cortex and exhibit specific stimulus selectivity, including face, color, object category, and surface properties (Tsao et al. 2003; Conway et al. 2007; Nishio et al. 2012; Sato et al. 2013). Horizontal axons in TE may project across different functional domains, and may exhibit projection pattern intermediate between inter-areal and intrinsic connections.

**Functional Implications of Anisotropic Distribution of Patches**

In adult macaque V1, horizontal projections from a certain site are often elongated across the cortical surface, perpendicular to ocular dominance stripes. This observation has been explained by the anisotropy of visual field cortical representation, imposed by interdigitating ocular dominance stripes (Amir et al. 1993; Lund et al. 1993; Yoshioka et al. 1996). In V1 of tree shrew and New World monkey, which contains weak or no ocular dominance stripes, the axis of elongation of horizontal projections in the retinotopic map corresponds to the preferred orientation of the projecting neurons (Bosking et al. 1997; Sincich and Blasdel 2001). In this study, we showed that the distribution of patches was already anisotropic even at 1 week after birth. This is reasonable because the formation of retinotopic, ocular dominance, and orientation maps, which are related to the anisotropic distribution of patches, does not require visual experience in carnivore V1 (see review by White and Fitzpatrick 2007), and ocular dominance columns are established in macaque V1 at this age (Rakic 1976; Huberman et al. 2005).

In TE, many injection cases exhibited a distribution of horizontal axon patches that was more drastically elongated along a preferred axis than that in V1 (Fig. 1). Although most of our materials were restricted to the gyral region of TE, we confirmed that the elongated distribution continued in the bank of the superior temporal sulcus in one case in which we examined deeper sections in the sulcus (Fig. 4). What are the functional implications of this anisotropic projection? As discussed earlier, TE consists of functionally different domains, each preferentially responding to different categories of objects and visual features (Tsao et al. 2003; Conway et al. 2007; Nishio et al. 2012; Sato et al. 2013). Horizontal axons may interconnect sites with a similar preference, and the anisotropic distribution of patches may reflect such functional domain structures. Further studies combining functional mapping and anatomical tracing may shed light on the functional role of horizontal axons in TE.

**Supplementary Material**

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

**Funding**

This study was supported by a grant for Core Research for Evolution Science and Technology (CREST) from Japan Science and Technology Agency, Special Coordination Fund for Promoting Science and Technology, and Grants-in-Aid for Scientific Research on Innovative Areas (15H01437 and 16H01673) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan, to I.F.; a Grant-in-Aid for Scientific Research (15K01851) from Japan Society for the Promotion of Science (JSPS), Japan, and a Grant for Promotion of Niigata University Research Projects (23B008), to H.T.

**Notes**

We thank Andreas Burkhalter, Guy N. Elston, Reuben H. Fan, Fuijo Murakami, Hiroshi Tamura, and Nobuhiko Yamamoto for their comments on earlier versions of the manuscript, and Leslie G. Ungerleider, Maree J. Webster, and Lynn Walker for their advice on anesthetic and surgical procedures in infant monkeys. We also thank the Cooperation Research Program of Primate Research Institute, Kyoto University for providing us with one of the animals used. Conflict of Interest: None declared.

**References**


Figure 11. Schematic drawing of postnatal development of terminal patches of horizontal axons in TE and V1. The striped circle at the center of each figure represents the injection site, the shaded circles connected to the injection site by lines represent terminal patches. Varying degrees of shading represent the varying connection densities between injection sites and patches. Discrete patches are present in both TE and V1 at 1 week of age (A,B); the size, interpatch distances, and distribution pattern are similar to those observed in adult monkeys (C,D). The connection density of patches continues to be refined after birth, in an area-specific manner in TE and V1.


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