Spinogenesis and Pruning from Early Visual Onset to Adulthood: An Intracellular Injection Study of Layer III Pyramidal Cells in the Ventral Visual Cortical Pathway of the Macaque Monkey

Neocortical pyramidal cells are characterized by markedly different structure among cortical areas in the mature brain. In the ventral visual pathway of adult primates, pyramidal cells become increasingly more branched and more spiny with anterior progression through the primary (V1), second (V2), and fourth (V4) visual areas and cytoarchitectonic areas TEO and TE. It is not known how these regional specializations in neuron structure develop. Here, we report that the basal dendritic trees of layer III pyramidal cells in V1, V2, V4, TEO, and TE were characterized by unique growth profiles. Different numbers of spines were grown in the dendritic trees of cells among these cortical areas and then subsequently pruned. In V1, V2, and V4, more spines were pruned than grew resulting in a net decrease in the number of spines in the dendritic trees following the onset of visual experience. In TEO and TE, neurons grew more spines than they pruned from visual onset to adulthood. These data suggest that visual experience may influence neuronal maturation in different ways in different cortical areas.

Keywords: cortex, dendrite, development, Hebbian rule, spines, spontaneous activity

Introduction

Pyramidal cell structure varies dramatically among cortical areas in the adult primate brain. Estimates of the total number of spines (putative excitatory inputs) in the dendritic trees of pyramidal cells reveal more than a 50-fold difference between populations of cells sampled in different cortical areas (Elston et al. 2006). There are systematic trends for increasingly more complex dendritic trees through series of functionally related cortical areas. For example, neurons become progressively larger, more branched, and more spiny with anterior progression through the dorsal and ventral visual pathways (Elston and Rosa 1997, 1998, 2000; Elston et al. 1999a, 1999b; Elston 2003c; Elston, Benavides-Piccione, Elston, DeFelipe, and Manger 2005a; Elston, Benavides-Piccione, Elston, Manger, and DeFelipe 2005a; Elston, Elston, Casagrande, and Kaas 2005b; Elston, Elston, Kaas, and Casagrande 2005). Similarly, there is a progressive systematic increase in complexity of pyramidal cell structure through somatosensory areas 3b, 1, 2, 5, and 7 (Elston and Rockland 2002; Elston, Benavides-Piccione, Elston, DeFelipe, and Manger 2005a; Elston, Benavides-Piccione, Elston, Manger, and DeFelipe 2005b) and with anterior progression through cingulate areas 23 and 24 to dorsolateral granular prefrontal cortex (Elston 2000; Jacobs et al. 2001; Elston, Benavides-Piccione, and DeFelipe 2005; Elston, Benavides-Piccione, Elston, DeFelipe, and Manger 2005c; Elston, Benavides-Piccione, Elston, Manger, and DeFelipe 2005c; Elston, Elston, Casagrande, and Kaas 2005a; Anderson et al. 2009).

These specializations in pyramidal cell structure are likely to influence cortical function at the systems, cellular and subcellular levels (Jacobs and Scheibel 2002; Elston 2003b; Treves 2005; Spruston 2008). Specifically, the complexity of their dendritic structure influences their biophysical properties, their functional capacity, and potential for plastic change (Koch 1999; Mel 1999; Jan YN and Jan LY 2001; Chklovskii et al. 2004; London and Häusser 2005). Differences in the number of spines in the dendritic trees, each of which receives asymmetrical excitatory synapse (DeFelipe et al. 1988; Petralia, Wang, Wenthold 1994a, 1994b; Petralia, Yokotani, Wenthold 1994; Arellano et al. 2007), reflect different numbers of inputs integrated by individual neurons and the complexity of patterns of connectivity. Indeed, there is a parallel in the complexity of pyramidal cell dendritic trees and various aspects of their physiological properties. For example, the increase in the size of the dendritic trees (in the tangential plane) parallels an increase in the receptive field size of neurons through the ventral pathway areas, the primary (V1), second (V2), and fourth (V4) visual areas, and cytoarchitectonic areas TEO and TE of the inferior temporal cortex (for reviews, see Elston 2002; Fujita 2002).

It remains to be determined how pyramidal cells come to be so specialized in different cortical areas. There are 2 opposing views in the literature regarding how cortical areas develop. One view posits that all cortical areas mature at the same rate (Bourgeois and Rakic 1993; Bourgeois et al. 1994), whereas the other view states that different cortical areas mature at different rates (Huttenlocher and Dabholkar 1997; Travis et al. 2005; Petanjek et al. 2008). Recently, we demonstrated that layer III pyramidal cells in V1, TE, and the granular prefrontal cortex of the macaque monkey grow, and subsequently prune, markedly different numbers of dendritic spines during normal development (Elston et al. 2009). Different numbers of spines are grown in the dendritic trees of these cells prior to birth. However, little is known about relative developmental profiles of pyramidal cells among cortical areas associated with functional hierarchies such as those proposed in visual cortex (Maunsell and Newsome 1987; Felleman and Van Essen 1991).

Here, we performed a systematic study in which we quantified the structure of layer III pyramidal cells in cortical areas of the ventral visual pathway of the macaque monkey from birth to adulthood. Our findings reveal similarities and differences in the developmental profiles of pyramidal cells among cortical areas. In particular, we reveal 2 opposing postnatal developmental trends: dendritic atrophy and net spine loss in V1, V2, and V4 and dendritic growth and net spinogenesis in TEO and TE.

Materials and Methods

Eight male cynomolgus monkeys (Macaca fascicularis) were used in the present study (Table 1). The cell injection methodology and
were incubated in a solution containing 10⁻⁵ M of the fluorescent dye 4,6-diamidino-2-phenylindole (DAPI; Sigma D9542, St Louis, MO) in PB at room temperature for 5–10 min and mounted between Millipore filters (AABG02500, Billerica, MA). The slice preparation was then mounted in paraformaldehyde in 0.1 M phosphate buffer (PB).

Serial thick sections (250 μm) were cut tangential to the cortical surface with the aid of a vibratome (Vibratome series 1000). Individual sections were incubated in a solution containing 1% hydrogen peroxide and 0.5% DAB in PB. This solution contains 2% bovine serum albumin (A3425; Sigma), 1% Triton X-100 (X100; Sigma-Aldrich), 0.1% sodium azide, and 5% sucrose in PB stock solution (2% bovine serum albumin [A3425; Sigma], 1% Triton X-100 [X100; Sigma-Aldrich], 0.1% sodium azide, and 5% sucrose in PB) for 4–11 days at room temperature, washed 3 times for 10 min each in PB, incubated in streptavidin-biotinylated horseradish peroxidase complex (1:100; RPN1051, GE Healthcare, Uppsala, Sweden) for 2 h, washed 3 times for 10 min each in PB, then incubated in 0.5% 3,3′-diaminobenzidine tetrahydrochloride (DAB, D5637, Sigma; 1:200 in PB) for 10 min at room temperature before being reacted in a solution containing 1% hydrogen peroxide and 0.5% DAB in PB. This method yields a light-stable robust reaction product (Fig. 2).

Cells were included for analysis only if they had an unambiguous apical dendrite, had their complete basal dendritic tree, and were well filled. Neurons were reconstructed with the aid of Neurolucida system (MBF Bioscience, Williston, VT) coupled with a microscope (Eclipse 80i; Nikon) equipped with a motorized stage (Ludl, Hawthorne, NY) and a CCD camera (CX9000; MBF Biosciences). The size of the dendritic trees was determined in the tangential plane as the area contained within a convex hull traced around the outermost distal dendritic terminations in reconstructions collapsed into 2 dimensions (Fig. 3 inset). The branching structure of the dendritic trees immunohistochemical processing employed in the present study have been outlined in detail in previous studies (Buhl and Schlote 1987; Elston and Rosa 1997; Elston 2001). The animals were deeply anesthetized with sodium pentobarbital (Nembutal, >75 mg/kg intravenously or intraperitoneally; Dainippon Sumitomo Pharma, Osaka, Japan) in accordance with protocols approved by Osaka University and regulations for the care and use of animals set out by the National Institutes of Health (publication No. 86-23, revised 1996) and perfused intracardially, and the brain was removed.

Tissue was taken from the exposed portion of the occipital lobe (V1; corresponding approximately to the central 5–7 degrees of the visual representation) (Daniel and Whitteridge 1961), the posterior bank of the inferior occipital sulcus (V2; corresponding approximately to the central 1–2 degrees) (Gattass et al. 1981; Levitt, Kiper, and Movshon 1994; Roe and T’so 1995), the middle third of the prelunate gyrus (V4; corresponding approximately to 10–20 degrees in the visual representation) (Gattass et al. 1988), the dorsolateral portion of the occipitotemporal transition (TEO), and the middle third of the inferior temporal gyrus immediately anterior to the posterior middle temporal sulcus (TE, TEP of Seltzer and Pandya 1978, TEPd of Yukie 1997) (Fig. 1). All tissue blocks were sampled from the right hemisphere. The white matter was trimmed from the blocks, and the remaining gray matter was "unfolded" and postfixed overnight between glass slides in a solution of 4% paraformaldehyde in 0.1 M phosphate buffer (PB).

In the present study, we focused on pyramidal cells at the base of layer III. For reasons outlined elsewhere (Casagrande and Kaas 1994; Elston and Rosa 1998), we use the nomenclature of Hassler (1966) for the cortical layer, thus avoiding confusion when comparing aspects of cortical microcircuitry among primate species and among cortical areas (e.g., Elston et al. 2006; Sherwood et al. 2007). Layer III was easily identified in the DAPI-labeled sections immediately above the neuron-dense granular layer. Even in tangential sections, it is easy to distinguish the transition from layer III to layer IV due to the change in density and size of somata (see Fig. 3 of Elston and Rosa 1997). For reasons outlined elsewhere (Casagrande and Kaas 1994; Elston and Rosa 1997), we use the nomenclature of Hassler (1966) for the cortical layer, thus avoiding confusion when comparing aspects of cortical microcircuitry among primate species and among cortical areas (e.g., Elston et al. 2006; Sherwood et al. 2007). Layer III was easily identified in the DAPI-labeled sections immediately above the neuron-dense granular layer. Even in tangential sections, it is easy to distinguish the transition from layer III to layer IV due to the change in density and size of somata (see Fig. 3 of Elston and Rosa 1997).

DAPI-labeled neurons were injected with Lucifer Yellow (Lucifer Yellow CH dilithium salt, L-0259, Sigma; dissolved in 0.05 M Tris buffer, pH = 8.4) under visual guidance with continuous current (up to 100 nA). Neurons were injected in tangential sections so as to be able to reconstruct the entire basal dendritic tree. By injecting neurons in the tangential plane, aspects of their structure can be related directly with features reported elsewhere such as intrinsic axon patches (Yoshioka et al. 1999). Such an approach has been central to the demonstration of regional and species specializations in pyramidal cell structure as the entire tangential extent of the basal dendritic tree is revealed, unlike in most previous studies in transverse sections in which many of the basal dendrites are truncated. Such studies in "thin" transverse sections bias for uniformity and mask the sort of differences in neuronal morphology detectable in tangential sections.

Once a suitable number of neurons had been injected, the slices were processed for a light-stable reaction product (Elston and Rosa 1997). The sections were processed in a solution containing 0.6 μg/ml biotinylated anti-Lucifer Yellow (A-5751; Invitrogen, Carlsbad, CA) in stock solution (2% bovine serum albumin [A3425; Sigma], 1% Triton X-100 [X100; Sigma-Aldrich], 0.1% sodium azide, and 5% sucrose in PB) for 4–11 days at room temperature, washed 3 times for 10 min each in PB, incubated in streptavidin-biotinylated horseradish peroxidase complex (1:100; RPN1051, GE Healthcare, Uppsala, Sweden) for 2 h, washed 3 times for 10 min each in PB, then incubated in 0.5% 3,3′-diaminobenzidine tetrahydrochloride (DAB, D5637, Sigma; 1:200 in PB) for 10 min at room temperature before being reacted in a solution containing 1% hydrogen peroxide and 0.5% DAB in PB. This method yields a light-stable robust reaction product (Fig. 2).

Table 1

<table>
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Note: 14M, 14 months of age; 23M, 23 months of age; N/A, not available.

Figure 1. Schematic illustrating where neurons were sampled (dots) in the primary (V1), second (V2), and fourth (V4) visual areas and cytoarchitectonic areas TEO and TE. The lateral view of the cerebral cortex is shown with the lunate sulcus (Is) and the inferior occipital sulcus (ios) opened and with a visuotopic map adapted from Gattass et al. (1981, 1988); pmts: posterior middle temporal sulcus.
number of branch points at 75 μm from the cell body or measure 1 for the spine density between 0 and 10 μm from the cell body, measure 2 for the spine density between 10 and 20 μm from the cell body, and measure 3 for the spine density between 20 and 30 μm from the cell body), and the data were tested by repeated measures analyses of variance (ANOVA). Statistical tests used here are the same as those used in our previous studies.

Results
A total of 955 pyramidal cells in layer III were included for analyses (Table 2). Over 130,000 individual dendritic spines were drawn and tallied. Data are reported as in previous studies of pyramidal cells in the adult brain (Elston and Rosa 1998; Elston et al. 1999a) to allow direct comparison of cell structure in the developing and adult visual cortex of the macaque monkey.

Basal Dendritic Field Areas
Pyramidal cells in V1, V2, V4, TEO, and TE differed in their growth profiles from postnatal day 2 (2D) to adulthood (Fig. 3). The dendritic trees of pyramidal cells in V1 were their largest at 2D and continued to diminish in size through 3 weeks of age (3W) to 3½ months of age (3½M), being 27% and 41% smaller than those at 2D, respectively. These cells then appeared to undergo a growth phase from 3½M to 7 months of age (7M) before achieving their adult size. Even in the adult, the dendritic trees of pyramidal cells in V1 were smaller by 34% than those observed at 2D. Cells in V2 and V4 were largest at 3½M, being 78% and 48% larger than those at 2D, respectively. Beyond 3½M, the dendritic trees of cells in V2 and V4 diminished to their adult size, being approximately the same size as observed at 2D. Cells in TEO and TE were largest in the adult brain, being approximately twice the size of those observed at 2D. Statistical analysis (one-way ANOVA) revealed that the differences in the size of the basal dendritic trees of pyramidal cells in any given cortical area were significant ($P < 0.05$) across the age groups (V1, $F_4 = 19.43$; V2, $F_4 = 67.00$; V4, $F_4 = 49.20$; TEO, $F_4 = 140.41$; TE, $F_4 = 100.06$).

Because of the different profiles observed in the size of the dendritic trees of cells in each cortical area, there were relative differences in the size of cells among cortical areas for any given age (Fig. 3). For example, at 2D, cells in V1 had larger dendritic trees than those in V2, TEO, and TE. By 3W, cells in V1 were smaller than those in other cortical areas, a pattern observed through to adulthood. At 3½M, cells in V4 and TEO had larger dendritic trees than those in area TE, whereas cells in TE are the largest in the adult brain. Statistical analysis (one-way ANOVA) revealed that the size of the dendritic trees of cells at any given age was significantly different ($P < 0.05$) among cortical areas in all animals (2D, $F_4 = 37.23$; 3W, $F_4 = 32.00$; 3½M, $F_4 = 112.28$; 7M, $F_4 = 137.44$; adult [AD], $F_4 = 311.12$).

Branching Patterns of the Basal Dendritic Trees
For any given cortical area, we found changes in the branching complexity, as evidenced by their Sholl profiles, with aging (Fig. 4). In V1, the peak branching complexity (defined as the maximum number of dendritic intersections with the Sholl annuli) occurred at 3W. The same was observed in V2 and TEO. In V4, however, the peak branching complexity occurred at 3½M. In TE, the peak branching complexity occurred at 7M. In addition, we found that the peak branching complexity within...
the dendritic trees of cells in a given cortical area may change in spatial distribution with aging. For example, in V1, the peak branching complexity was located 75 μm from the cell body at 2D but at 50 μm from the cell body at 3W, 3½M, and 7M. In V4, the peak branching complexity was located 50 μm from the cell body at 2D and 3W but at 75 μm from the cell body at 3½ and 7M. Statistical analysis (1-way repeated measures ANOVA) revealed that the number of dendritic intersections with Sholl annuli within any given cortical area was significant \((P < 0.05)\) across the age groups (V1, \(F_{4} = 4.97\); V2, \(F_{4} = 5.38\); V4, \(F_{4} = 11.21\); TEO, \(F_{4} = 3.71\); TE, \(F_{4} = 13.58\)). Comparison of the branching complexity in the dendritic trees of pyramidal cells among cortical areas at each given age revealed that cells in V1 and V2 have fewer branches than those in TEO and TE at all corresponding ages (Fig. 4; \(P < 0.05\), Mann-Whitney \(U\)-test).

Statistical analysis (one-way repeated measures ANOVA) revealed that the number of dendritic intersections with Sholl annuli was significantly different among cortical areas \((P < 0.05)\) at each given age (2D, \(F_{7} = 13.00\); 3W, \(F_{7} = 11.48\); 3½M, \(F_{7} = 33.07\); 7M, \(F_{7} = 45.77\); A, \(F_{7} = 51.20\)).

**Spine Densities of the Basal Dendrites**

In all cortical areas, we found a trend for increasing spine density from 2D through 3W to 3½M (Fig. 5; see also Fig. 2 for microphotographs). This increase was evident along the entire extent of the dendrites, with the exception of the proximal region, which is devoid of spines. By 7M, there had been a considerable decrease in spine density in the dendritic trees of cells in all cortical areas. Again, for any given cortical area, this decrease in spine density was relatively uniform along the entire extent of the dendrite (with the exception of the proximal segments). There were, however, notable differences in the rate of increase/decrease in spine density, as well as the peak spine density (the highest value observed along the entire extent of the dendrites), among cortical areas at the different age groups. For example, although cells in V1 have a lower spine density profile than those in V2 at 2D, the rate of increase in spine density in V1 with aging exceeds that in V2 such that by 3½M cells in V1 have markedly higher spine density than those in V2. A similar trend was observed between V1 and V4. The highest rates of increase in spine density were observed in TEO and TE. Spine density in area TEO increased by 2-fold at comparable distances along the dendrites from 3W to 3½M. Repeated measures ANOVAs revealed these differences in spine density in each cortical area to be significantly different \((P < 0.05)\); V1, \(F_{4} = 417.4\); V2, \(F_{4} = 441.9\); V4, \(F_{4} = 609.5\); TEO, \(F_{4} = 594.5\); TE, \(F_{4} = 300.1\)). Repeated measures ANOVAs also revealed significant differences \((P < 0.05)\) in spine density.
among cortical areas for each given age group (2D, $F_4 = 243.2$; 3W, $F_4 = 456.1$; 3½M, $F_4 = 287.4$; 7M, $F_4 = 375.5$; A, $F_4 = 169.7$).

By combining data from the Sholl analyses with that of spine densities, we were able to calculate an estimate for the total number of dendritic spines in the basal dendritic tree of the “average” pyramidal neuron in each area for the different age groups (Elston 2001). These calculations revealed dramatic differences in spinogenesis and pruning in the dendritic trees of cells in visual cortex (Fig. 6). Cells in V1 had approximately 1900 spines in their dendritic trees at 2D; this number increased to a maximum of approximately 3800 at 3½M and subsequently decreased to approximately 900 in the adult. Cells in V2 had an estimated 2800 spines in their dendritic trees at 2D, increasing to a maximum of nearly 5000 at 3½M before declining to 1100 in the young adult. Cells in V4 were the most spinous of all cells at 2D (approximately 9000). Interestingly, cells in V4 then appeared to prune spines in the following weeks before undergoing considerable spinogenesis to reach a maximum of >9000 spines at 3½M. Thereafter, spines were pruned to reach 2400 spines in the adult. Cells in TEO had 3600 spines at 2D, increasing to 14100 spines at 3½M before decreasing to 4800 spines in the young adult. Cells in TE had 3100 spines at 2D, increasing to nearly 10400 spines at 3½M before decreasing to 6200 spines in the adult. In V1, V2, and V4, cells in the adult are considerably less spiny than those at 2D.

### Table 2

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Note: 14M, 14 months of age; 23M, 23 months of age.

Data for V1 (except for those from C12) and TE are shared with a previous study (Elston et al. 2009).
meaning that more spines were pruned from the dendritic trees of these cells than are grown. In TEO and TE, on the other hand, cells in the adult are considerably more spiny than those at 2D. Thus, in inferior temporal cortex spine growth is greater than spine loss, resulting in a net increase in spine number.

**Somal Areas**

Individual cell bodies were drawn, and the somal area was measured in the plane tangential to the cortical layers (Fig. 7). In V1, the largest cell bodies were observed at 3W, being slightly larger than at 2D. The cell bodies were smaller in older infants, being 43% and 34% smaller than the maximum at 3½ and 7M, respectively. Even in the adult, the cell bodies of pyramidal cells in V1 were >40% smaller than those observed at 2D. Likewise, in V2, the largest cell bodies were observed at 3W. The cell bodies became progressively smaller through 3½ and 7M to adulthood. In V4, the largest cell bodies were observed at 3½M, being 56% larger than those at 3W. In TEO and TE, there was a trend for increasingly larger cell bodies from 2D to adulthood. Statistical analysis (one-way ANOVAs) revealed that the size of the cell bodies of pyramidal cells in any given cortical area was significantly different ($P < 0.05$) across the age groups (V1, $F_4 = 72.42$; V2, $F_4 = 35.06$; V4, $F_4 = 41.47$; TEO, $F_4 = 34.24$; TE, $F_4 = 22.24$).

Comparison of the size of the cell bodies among cortical areas at each given age revealed variation in interareal trends with aging (Fig. 7). Cells in V4 had the largest cell bodies at most ages (2D, 3½ months, and adult). At 3W and 7M, cells in area TE had the largest cell bodies. Statistical analysis (one-way ANOVAs) revealed that the size of the cell bodies at any given age was significantly different among cortical areas ($P < 0.05$) in all animals (2D, $F_4 = 56.02$; 3W, $F_4 = 5.06$; 3½M, $F_4 = 81.21$; 7M, $F_4 = 66.54$; A, $F_4 = 62.93$).

**Discussion**

In the present investigation, we studied layer III pyramidal cell structure in the primary (V1), second (V2), and fourth (V4) visual areas as well as cytoarchitectonic areas TEO and TE of the inferior temporal cortex in monkeys ranging in age from 2 to 7 months.
Spinogenesis and Pruning in the Ventral Pathway

Reported a general trend for increasing surround suppression (maximum) increases from birth to adulthood. They also frequency at which cells give a response of at least 10% of its spatial resolution of cells in V1 (the highest spatial.

Movshon et al. (1999, 2000) demonstrated that receptive field size, spatial acuity, and surround suppression could plausibly be attributed to changes in the geometrical sampling of neurons as their dendritic trees change shape (cf., Sholl 1955; Ferster 1998; Taylor et al. 2000; Vaney and Taylor 2002). Specifically, as the dendritic trees of neurons decrease in size with maturation, they may sample a progressively smaller portion of the visuotopically organized afferent projection and thus have progressively smaller receptive fields. Additionally, by sampling a smaller proportion of the topographic map cells in the adult have increased spatial resolution as defined by their geometrical sampling strategies (for reviews on sampling geometry, see Sholl 1956; Malach 1994; Elston 2003a). However, although consistent with this interpretation, the decrease in the size of the dendritic trees of pyramidal cells cannot account fully for the decrease in the size of their receptive fields (65% and 41%, respectively). Quite possibly, pruning of afferent fibers to V1 during this time (Lund et al. 1977) may account for this difference, although this remains to be determined.

Zhang et al. (2008) reported that in V2, receptive fields of neurons mature later than those in V1 neurons, consistent with our finding that the dendritic trees of pyramidal cells in V2 continue to grow from 2D to 3½M, whereas those in V1 become smaller during this time. Neurons in area TE are not visually responsive until even later in development—about 4 months of age (Rodman et al. 1994). Anatomical connections of TE undergo a protracted period of refinement from birth to adulthood compared with other visual areas (Webster et al. 1991, 1994, 1995; Rodman and Consuelos 1994; Barone et al. 1996; Coogan and Van Essen 1996), and this region becomes myelinated much later in development than V1 (cf., Rodman 1994). Based on the present data, which reveal progressively larger dendritic trees in TE and TE neurons from birth to adulthood, it might be reasonable to assume that the receptive fields of neurons in inferior temporal cortex become increasingly larger with aging. Longitudinal quantitative studies will be required to provide the required empirical data.

Branching Complexity

Although the dendritic trees of pyramidal cells in V1 were at their largest at 2D, they were most branched at 3W. This age disparity may be attributable to the different functional tasks achieved by size versus branching complexity in the dendritic tree. Although we have discussed above how the size of the dendritic trees may influence the receptive field size of the neuron, branching structure may be important in determining orientation and direction selectivity in V1 (cf., Pettigrew 1974; Tieman and Hirsch 1982; Elston and Rosa 1997; Ferster 1998; Livingstone 1998). Whereas 10-day-old monkeys are already capable of color discrimination, form discrimination only becomes discernable at 3W (Zimmermann 1961). Is it a coincidence that the onset of form discrimination occurs at the time when cells are most branched? Could the increase in the number of branches facilitate compartmentalization of processing within the dendritic tree (Poirazi and Mel 2001; Chklovskii et al. 2004) and allow detection of inputs associated with asymmetric features throughout the dendritic tree?

The temporal sequence of attaining maximum size then maximum branching complexity observed in V1 was not characteristic of extrastriate cortical areas. The greatest

Figure 6. Graph of the total number of spines in the basal dendritic tree of the "average" layer III pyramidal cell in V1, V2, V4, TEO, and TE at 2D, 3W, 3½M, 7M, and adults (AD). Error bars = standard errors.

Size of the Dendritic Trees and Receptive Field Size

Our data in V1, which reveal a decrease in the size of the dendritic trees of pyramidal cells from early in postnatal development to adulthood, are consistent with the data of Boothe et al. (1979) who demonstrated that the basal dendrites of layer III pyramidal cells are longer early in development than in the adult, although their data on stellate cells suggest that maximal dendritic size might be achieved at slightly different ages among cell types and/or cortical layers. These anatomical data are consistent with various aspects of cell function. Movshon et al. (1999, 2000) demonstrated that receptive field sizes of neurons located in the central 5 degrees of the visual representation in V1 decrease in size from early postnatal development to adulthood. In addition, they demonstrated that the spatial resolution of cells in V1 (the highest spatial frequency at which cells give a response of at least 10% of its maximum) increases from birth to adulthood. They also reported a general trend for increasing surround suppression from birth to adulthood (for reviews, see Kiorpes and Movshon 2003; Chino et al. 2004). These changes in receptive field size, spatial acuity, and surround suppression could plausibly be attributed to changes in the geometrical sampling of neurons as their dendritic trees change shape (cf., Sholl 1955; Ferster 1998; Taylor et al. 2000; Vaney and Taylor 2002). Specifically, as the dendritic trees of neurons decrease in size with maturation, they may sample a progressively smaller portion of the visuotopically organized afferent projection and thus have progressively smaller receptive fields. Additionally, by sampling a smaller proportion of the topographic map cells in the adult have increased spatial resolution as defined by their geometrical sampling strategies (for reviews on sampling geometry, see Sholl 1956; Malach 1994; Elston 2003a). However, although consistent with this interpretation, the decrease in the size of the dendritic trees of pyramidal cells cannot account fully for the decrease in the size of their receptive fields (65% and 41%, respectively). Quite possibly, pruning of afferent fibers to V1 during this time (Lund et al. 1977) may account for this difference, although this remains to be determined.

Zhang et al. (2008) reported that in V2, receptive fields of neurons mature later than those in V1 neurons, consistent with our finding that the dendritic trees of pyramidal cells in V2 continue to grow from 2D to 3½M, whereas those in V1 become smaller during this time. Neurons in area TE are not visually responsive until even later in development—about 4 months of age (Rodman et al. 1994). Anatomical connections of TE undergo a protracted period of refinement from birth to adulthood compared with other visual areas (Webster et al. 1991, 1994, 1995; Rodman and Consuelos 1994; Barone et al. 1996; Coogan and Van Essen 1996), and this region becomes myelinated much later in development than V1 (cf., Rodman 1994). Based on the present data, which reveal progressively larger dendritic trees in TE and TE neurons from birth to adulthood, it might be reasonable to assume that the receptive fields of neurons in inferior temporal cortex become increasingly larger with aging. Longitudinal quantitative studies will be required to provide the required empirical data.

Figure 6. Graph of the total number of spines in the basal dendritic tree of the "average" layer III pyramidal cell in V1, V2, V4, TEO, and TE at 2D, 3W, 3½M, 7M, and adults (AD). Error bars = standard errors.
branching complexity in the dendritic trees of neurons in V2 was found at 3W, a time when these cells are at their maximum size. Coincident maximum size and maximum branching complexity was also observed in V4 but at 3½M. Cells in TEO and TE attain their greatest branching complexity at 3W and 7M, respectively; yet these cells are characterized by continual dendritic growth from 2D to adulthood. These data suggest that factors mediating the size and branching complexity of the dendritic trees of layer III pyramidal cells may be independent of each other or may interact differently among cortical areas (e.g., Ruchhoeft et al. 1999; Li et al. 2000; Nakayama et al. 2000; Tashiro et al. 2000; Wong et al. 2000; Arendt et al. 2004).

Spinogenesis and Pruning
There are differences in opinion regarding the time course of in vivo spinogenesis, synapse formation, and pruning in the cerebral cortex (Rakic et al. 1986; Huttenlocher and Dabholkar 1997). On the one hand, it has been suggested that synaptogenesis occurs concurrently among cortical areas, whereas on the other hand, it has been suggested that synaptogenesis occurs sequentially (for reviews, see Huttenlocher and Dabholkar 1997; Rakic and Kornack 2001; Guillery 2005). These theories derive from the quantification of the number of synapses per unit neuropil and are not corrected for cell death. Data leading to the former view were obtained from the macaque monkey (Rakic et al. 1986; Bourgeois and Rakic 1993), whereas those supporting the later view were obtained from the human brain (Huttenlocher 1979; Huttenlocher et al. 1982; Huttenlocher and de Courten 1987; Travis et al. 2005).

The present data confirm and expand the former view for the macaque monkey. Specifically, the present data reveal that peak spine density was attained in V1, V2, V4, TEO, and TE at approximately 3½ M. (Note that animals were only sampled at 2 D, 3 W, 3½ M, 7 M, and 4½ Y.) However, the present observations add a new twist to the story: peak spine density varied in magnitude among cells in the different cortical areas (Fig. 5). Furthermore, our estimates of the total number of spines within the dendritic trees of pyramidal cells varied by >3½-fold among cortical areas at the time of peak spine density, due to differences in the size and branching structure of the dendrites (3900 spines in V1 and 14 100 spines in TEO). In addition, there was a net decrease in the number of spines (putative excitatory synapses) within the dendritic trees of layer III pyramidal cells from visual onset to adulthood, as is the case in V1, V2, and V4. This trend, however, is not true for all cortical areas. In TEO and TE, it appears as though more spines are grown from visual onset to 3½M that are then subsequently pruned during maturation to adulthood. A much higher
proportion of spines are pruned from the dendritic trees of neurons in V1 (approximately 80%), V2 (77%), and V4 (73%) than in areas TEO and TE (41% and 46%, respectively) from 3½M to adulthood. It remains to be determined whether these trends are characteristic of other neurons in these same cortical areas or if they are specific to layer III pyramidal cells. We have begun a study of infragranular pyramidal cells to investigate this further.

Conclusions
Here, we demonstrated that the dendritic trees of pyramidal cells in V1, V2, V4, TEO, and TE have different growth and atrophy profiles during normal development. We found differences in the timing and rate that branches are grown and pruned from within the dendritic trees among cortical areas. In all cortical areas, we found a trend for increasing spine density along the dendrites from birth to approximately 3½M, beyond which pruning exceeded any further spine growth resulting in a net decrease in the number of spines found within the dendritic trees. The extent of this pruning differed among cortical areas; cells in V1, V2, and V4 actually prune more spines following visual onset than they grow, whereas cells in TEO and TE do not. The result is that cells in the adult V1, V2, and V4 have fewer spines than they are born with, whereas cells in adult TEO and TE have as many as 2-fold the number of spines they are born with. To better comprehend the structural correlates of development and maturation of visual behavior, it is clearly necessary to expand the scope of investigation of developmental cell physiology beyond visual areas of the occipital lobe.

Funding
Japan Science and Technology Agency (Core Research for Evolutional Science and Technology) to I.F. and G.N.E.; Ministry of Education, Culture, Sports, Science, and Technology (Japan) (17022025 to I.F.); Osaka University to I.F.; I Hear Innovation Foundation (Australia) to G.N.E.

Notes
We thank Noritaka Ichinohe for his comments on the manuscript. Conflict of Interest: None declared.
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