The Journal of Neuroscience, May 1990, 10(5): 1407–1414

Correlations Between Morphology and Electrophysiology of Pyramidal Neurons in Slices of Rat Visual Cortex. I. Establishment of Cell Classes

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The purpose of this study was to determine whether different classes of neocortical pyramidal neurons possess distinctive electrophysiological properties. Neurons were assigned to classes on the basis of their somadendritic morphology as revealed by intracellular HRP injection. The electrophysiological features of each class are compared in the following paper (Mason and Larkman, 1990).

Coronal slices were prepared from the visual cortex of the albino rat and maintained *in vitro*. Pyramidal neurons from layers 2/3 and 5 were impaled with electrodes containing HRP. After intracellular recording, the cells were injected, reacted to visualize the enzyme, and examined in the light microscope. The 18 neurons from layer 2/3 showed an underlying similarity of somadendritic morphology and were considered to constitute a single cell class, although some features varied with the depth of the soma in the cortex. The 22 layer 5 cells were more diverse and were divided into 2 main classes. Eleven cells had single, thick apical trunks which ascended to close to the layer 1 border then branched to form a terminal arbor. These cells were termed thick layer 5 cells, and were found mainly in the upper part of the layer. Ten layer 5 cells had single apical trunks which tapered to a fine diameter and terminated, without forming an obvious terminal arbor, near the top of layer 4 (3 cells) or in layer 2/3 (7 cells). These cells were termed slender layer 5 cells and were found throughout the layer. One layer 5 cell, which had an apical trunk that bifurcated near the top of layer 4 and ascended as 2 parallel trunks through layer 2/3, could not be included in either of these classes and was not analyzed further.

Somadendritic morphology was studied quantitatively, and differences were found between the 3 classes for several features. Slender layer 5 cells had less elongate somata and longer basal and oblique dendrites than the other cell classes. Thick layer 5 cells had greater combined dendritic lengths and dendritic shaft membrane areas than other classes. Comparison with previous studies indicated that the HRP injection technique permitted a more complete visualization of dendritic arbors than Golgi-based methods. It was found that the basal and oblique dendrites together contributed a high proportion (often over 70%) of the total neuronal shaft membrane area.

In common with other neocortical areas, the visual cortex contains neurons with a wide variety of morphologies (Peters and Jones, 1984). Neocortical neurons can be broadly categorized as pyramidal or nonpyramidal on the basis of their somadendritic morphology, but there is much variation and many subdivisions are possible within these 2 categories. Pyramidal neurons are the predominant output neurons of the neocortex, accounting for 70–90% of the total neuronal population of the visual cortex (Peters, 1987). Given the laminar organization of neocortex, with different classes of intrinsic and extrinsic afferents terminating in specific layers (Gilbert, 1983; Burkhalter, 1989), the dendritic morphology of a neuron will influence the spectrum of inputs available to it. Additionally, cells in different layers may have different cortical or subcortical projection targets (Nauta and Bucher, 1954). Some of the variation in pyramidal dendritic morphology is related to the laminar location of the soma, but it is becoming clear that even within a single layer, cells may have distinctive dendritic patterns which correlate with their projection targets (Katz, 1987; Hübener and Bolz, 1988). Neocortical pyramidal neurons also show variation in their intrinsic electrophysiological properties (Calvin and Sypert, 1976; McCormick et al., 1985), but the question remains to what extent particular electrophysiological features are restricted to cells of a specific morphology. This question is of interest, because if different morphological classes have distinctive intrinsic electrophysiological properties, it suggests the existence of specific "types" of pyramidal cell specialized for particular roles within the cortex. In order to address this question, we have embarked on a study of the somadendritic morphology and intrinsic electrophysiology of neurons of the visual cortex of the rat, using a combination of intracellular recording, dye injection, and *in vitro* slice techniques (Larkman et al., 1988). Rodent visual cortex is similar in many respects to that of cats and monkeys in terms of its functional architecture, microcircuitry, and the response properties of individual neurons (Tiao and Blakemore, 1976; Diao et al., 1983; Bode-Gruel et al., 1987; Burkhalter, 1989). In this paper we describe the morphological features of pyramidal cells from layers 2/3 and 5 and use these features to establish cell classes. In the following paper (Mason and Larkman, 1990), we compare the intrinsic electrophysiological properties shown by these different classes.

Received June 14, 1989; revised Oct. 2, 1989; accepted Nov. 13, 1989.

This work was supported by The Wellcome Trust (grant 15932/1.5) and the Medical Research Council (grant PG7900491). A.L. is a Beit Memorial Research Fellow. We are grateful to Ken Stratford for assistance with computer programming.

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Materials and Methods

Slice preparation and maintenance. Thirty-one male Wistar rats, ranging in weight from 130 to 160 gm, were used in this study. Full details of slice preparation and maintenance are given in Larkman et al. (1988). In brief, 3 or four 400-μm-thick coronal slices were prepared from the occipital cortex of one hemisphere. The slices were trimmed with a razor blade to consist entirely of visual cortex, as determined from published stereotaxic and cytoarchitectonic studies (Zilles et al., 1984). Each slice was then bisected orthogonal to the pial surface, so that each piece was about 1.5 mm wide. The slices were maintained in an interface-type recording chamber at 34.5 ± 0.5°C. Slices remained in the chamber for 2 hr before intracellular impalements were attempted. The composition of the artificial cerebrospinal fluid (ACSF) used throughout slice preparation and electrophysiological recording was (in mM) NaCl, 124; KCl, 3.3; $MgSO_4$, 1; KH_2PO_4, 1.3; $CaCl_2$, 2.5; $NaHCO_3$, 26; glucose, 10; pH 7.4.

HRP injection. Micropipettes were usually filled with a solution of 1 or 2% HRP (Type VI, Sigma) in 0.5 M KCl and had resistances of 40–100 MΩ. For some experiments 1.0 M KCl or 0.5 M KAc was used in place of 0.5 M KCl. Although cortical layers could not be distinguished in the slice chamber, micropipettes were positioned approximately with the aid of a binocular microscope equipped with an eyepiece graticule. Accurate determination of the cortical layers and the position of the HRP-injected cells was performed later on the fixed slices. The micropipettes were advanced through the brain slice by a microdrive with position display (Digitimer, SCAT-01). Neuronal impalements were accepted only at depths greater than 100 μm and less than 300 μm within the slice. After electrophysiological recording, the cells were injected with HRP using a pressure injection technique (Mason et al., 1988). After injection, the micropipette was withdrawn from the cell, and the HRP was allowed to spread for a period before the slice was removed from the chamber and fixed. For layer 2/3 cells, the spread time was 20–40 min, whereas for layer 5 cells it was usually 60–90 min.

Histological procedures. Slices were fixed by immersion in 2.5% glutaraldehyde and 1% formaldehyde in 0.1 M phosphate buffer, pH 7.4, for 2 hr followed by an overnight rinse in the same buffer. The slices were then resectioned at 60 μm thickness using a vibrating microtome and reacted to visualize the HRP as described previously (Larkman et al., 1988). Briefly, the sections were reacted with Hanker-Yates reagent and hydrogen peroxide (Hanker et al., 1977), osmicated, dehydrated using ethanol, and embedded in epoxy resin between slides and coverslips.

Morphological analysis. The terminology employed for dendritic trees is modified after that of Percheron (1979). Dendrites branch at *branch points,* which in our sample were always binary; no clear examples of trifurcations were recorded. The portion of a dendrite between 2 branch points, between the soma and a branch point, or between a branch point and the end of a dendrite is called a dendritic *segment.* The termination of a dendrite is called its *tip,* and a segment ending in a tip is a *terminal* segment. Dendritic segments adjoining the soma are termed *stem* segments. The apical dendrite of a pyramidal cell consists of a *trunk,* which may give off *oblique* branches of lesser diameter before dividing to form a *terminal arbor.*

The cells were reconstructed from camera lucida drawings made at × 1000 magnification using an × 100 oil-immersion objective (n.a. 1.25), and the branching patterns of the basal and apical arbors of each cell were determined. The lengths and diameters of each dendritic segment were measured using an eyepiece graticule at a magnification of × 1875. The diameter of each segment (not including dendritic spines) was measured at 3 points along its length and the average diameter recorded. Dendritic segments that showed substantial taper were subdivided. All segments were considered to be circular in cross-section. Dendritic length measurements were corrected for 3-dimensional projection errors using depth values obtained from the calibrated fine-focus control of the microscope, according to Pythagoras's theorem. The branching pattern, length, and diameter measurements were entered into a computer program which calculated the combined dendritic length and membrane area for each dendritic arbor. Means and standard deviations have been quoted for descriptive purposes, but for further analysis methods were used that did not require the assumption that the data was normally distributed. Comparisons between classes were made using the Mann-Whitney U-test, and the Kendall coefficient of rank correlation (tau) was used to test for correlations in the data.

Tissue shrinkage. By adopting an electron microscopy-based fixation and embedding strategy, and in particular by osmicating the sections prior to dehydration and by embedding in epoxy resin, it was hoped that tissue shrinkage would be kept to a minimum. The thickness of the cortical gray matter was measured for each of our preparations, and a value of 1.49 mm (±0.05, $n = 41$) was obtained. This is identical to the value obtained by Peters et al. (1985) for rat visual cortex, both before and after tissue preparation using fixation and embedding procedures similar to ours. These authors concluded that tissue shrinkage under such conditions was minimal (< 1%), and a recent study of rat hippocampus has reached a similar conclusion (West et al., 1988). However, other authors have reported and corrected for substantial shrinkage in similar studies (e.g., Beaulieu and Colonnier, 1983). Shrinkage may affect various components of the tissue unequally, and the processes of injected and filled cells may corrugate rather than reducing in length if shrinkage occurs. In view of these complexities, we have not applied a correction for shrinkage in this study, but the possibility that dimensions have been underestimated as a result must be considered.

Results

Qualitative morphology

This analysis is based on a sample of 40 pyramidal cells located in layers 2/3 and 5 which were judged to be completely filled and had all parts of the dendritic arbor clearly visible. An additional 15 cells were recovered whose morphological appearance was deficient in some respect, but which could still be assigned to a category with confidence.

Layer 2/3 cells

The 18 layer 2/3 (L2/3) cells were distributed throughout the thickness of layer 2/3, with the exception of the extreme lower portion. No boundary between layers 2 and 3 could be discerned histologically, but if an arbitrary division was placed at half the depth of 2/3, the sample consisted of 6 layer 2 cells and 12 from layer 3.

Most L2/3 cells had a pyramidal- shaped soma, with a skirt of basal dendrites emerging around the lower part. Rarely, one or more basal dendrites arose from the upper part of the soma. Most basal dendrites branched repeatedly close to the soma, but dendrites that did not branch from soma to tip were occasionally seen. All cells except one had a single apical trunk arising from the upper surface of the soma and ascending toward the pia, giving off a variable number of oblique branches as it did so. The pattern of branching of the apical dendritic arbor was variable between cells, and some of this variation was related to the depth of the soma within the cortex (Fig. 1). The apical trunks divided close to the upper boundary of layer 2/3 to form a terminal arbor in layer 1, so the length of the trunk varied with the depth of the soma in the cortex, deep cells having longer trunks than more superficial cells. Superficial cells also had more extensive, more highly branched terminal arbors than deeper cells. Our sample contained one cell whose soma was located at the extreme upper limit of layer 2 and which had no apical trunk. Instead, 2 ascending dendrites emerged from the upper surface of the soma and arborized in layer 1 (Fig. 1*A*).

Layer 5 cells

The layer 5 (L5) cells appeared to be more heterogeneous than the L2/3 cells, particularly with respect to the pattern of branching of the apical dendritic arbor.

Eleven cells could be distinguished by having relatively thick apical trunks which gave rise to obvious terminal arbors in layer 1. These cells will be termed thick L5 cells, and an example is shown in Figure 2*A*. They had vertically elongate somata which tapered gradually towards the thick bases of the apical dendrites. The apical dendrites ascended and gave off a relatively large

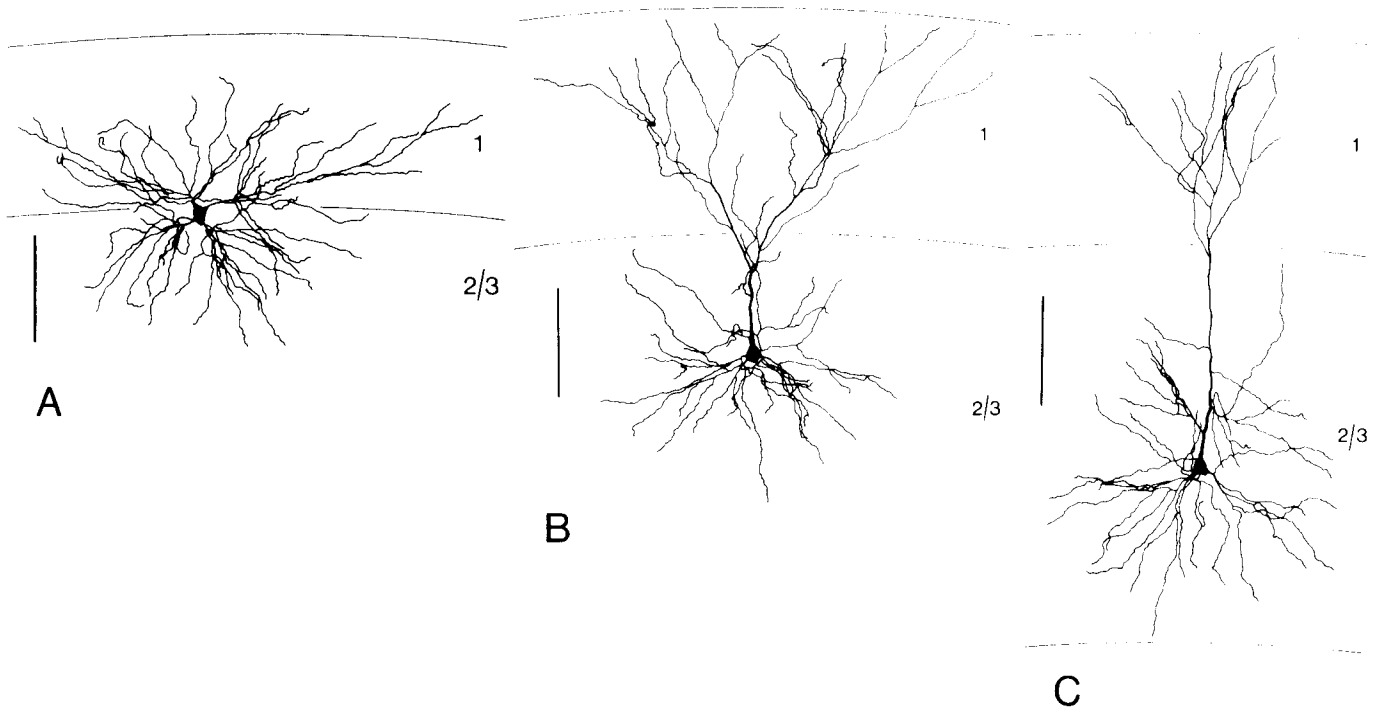

Figure 1. Pyramidal neurons from layer 2/3. Camera lucida reconstructions of the somata and dendrites of 3 cells from different depths in layer 2/3, showing the range of morphologies encountered. *A*, Superficial pyramidal cell with its soma close to the layer 1 boundary. This cell has no apical trunk and 2 dendrites emerge from the upper part of the soma and form arbors in layer 1. *B*, Cell from the upper part of layer 2/3, with the short apical trunk and widespread terminal arbor typical of cells from this depth. *C*, Cell from the lower part of 2/3, with a longer apical trunk and less widespread terminal arbor than *B*. In this and Figure 2 the dendritic spines, which cover all but the most proximal parts of the dendrites, have not been shown. Scale bars, 100 μm.

number of oblique branches, mainly within layer 5. They passed through most of layer 2/3 showing little tapering and giving off few if any oblique branches, to divide near the layer 1 boundary and form a terminal arbor in layer 1. This pattern of branching was very consistent between cells in this group. Thick L5 cells were found mainly in the upper part of the layer.

Ten L5 cells had slender, tapering apical trunks which did not form visible terminal arbors in layer 1, and were termed slender L5 cells (Fig. 2, *B*, *C*). These cells had squat somata from which the apical dendrite emerged abruptly. The branching pattern of the basal dendrites was more variable than in the other cell classes. In one cell, the basal arbor was not radially symmetrical but appeared biased to one side of the soma, and in 2 cells there were some basal dendrites substantially longer than the majority. The apical dendritic arbors were also variable. The initial part of the apical dendrite was often thick, but soon tapered. Oblique branches varied considerably in number but were generally less numerous than for thick L5 cells. The more distal part of the apical dendrite was slender and either terminated, without arborizing, near the top of layer 4 (3 cases; Fig. 2*B*) or ascended as an extremely fine process to terminate, also without arborizing, in layer 2/3 (Fig. 2*C*). Some cells showed one or more dendritic branches near the distal end of the apical dendrite. These were thought to be oblique branches rather than part of a terminal arbor because in every case a continuation of the apical trunk could be distinguished which was clearly longer than any other branch. Five of the 7 cells with apical dendrites extending into layer 2/3 had displaced cell bodies (Miller, 1988), in which the soma is aligned along a different axis to the apical dendrite. Cells of other types did not display this characteristic. Slender cells were found throughout the depth of layer 5, with the exception of the extreme lower portion, where injections were not attempted.

One cell could be distinguished clearly from the preceding classes because its apical dendrite bifurcated in layer 4, giving rise to 2 apical trunks of equal diameter which ascended through layer 2/3 closely parallel to each other (not illustrated). Because we obtained only a single example, more detailed analysis of this cell was not performed.

Quantitative morphology

The somadendritic morphology of all the neurons in the sample except the cell with the bifurcating apical trunk was studied quantitatively.

Diameter of apical dendrite

The diameter of the apical dendrite has previously been employed as a measure to distinguish between different types of layer 5 pyramidal cells (Peters and Kara, 1985). We measured the apical trunk diameter of the thick and slender L5 cells at 100-μm intervals between 100 and 700 μm from the soma. Proximally, there was some overlap in the apical trunk diameters between the 2 classes, but by 300 μm from the soma they became nonoverlapping (Fig. 3). At all distances measured, the 2 classes were significantly different. At the level of upper layer 4, the mean diameter of the apical dendrites of the thick L5 cells was 2.1 ± 0.6 μm, and for slender cells was 0.6 ± 0.2 μm.

Soma size and shape

For each cell the somal height (measured along a line orthogonal to the pial surface) and width (parallel to the pial surface) were measured. From Table 1 it can be seen that the somal heights

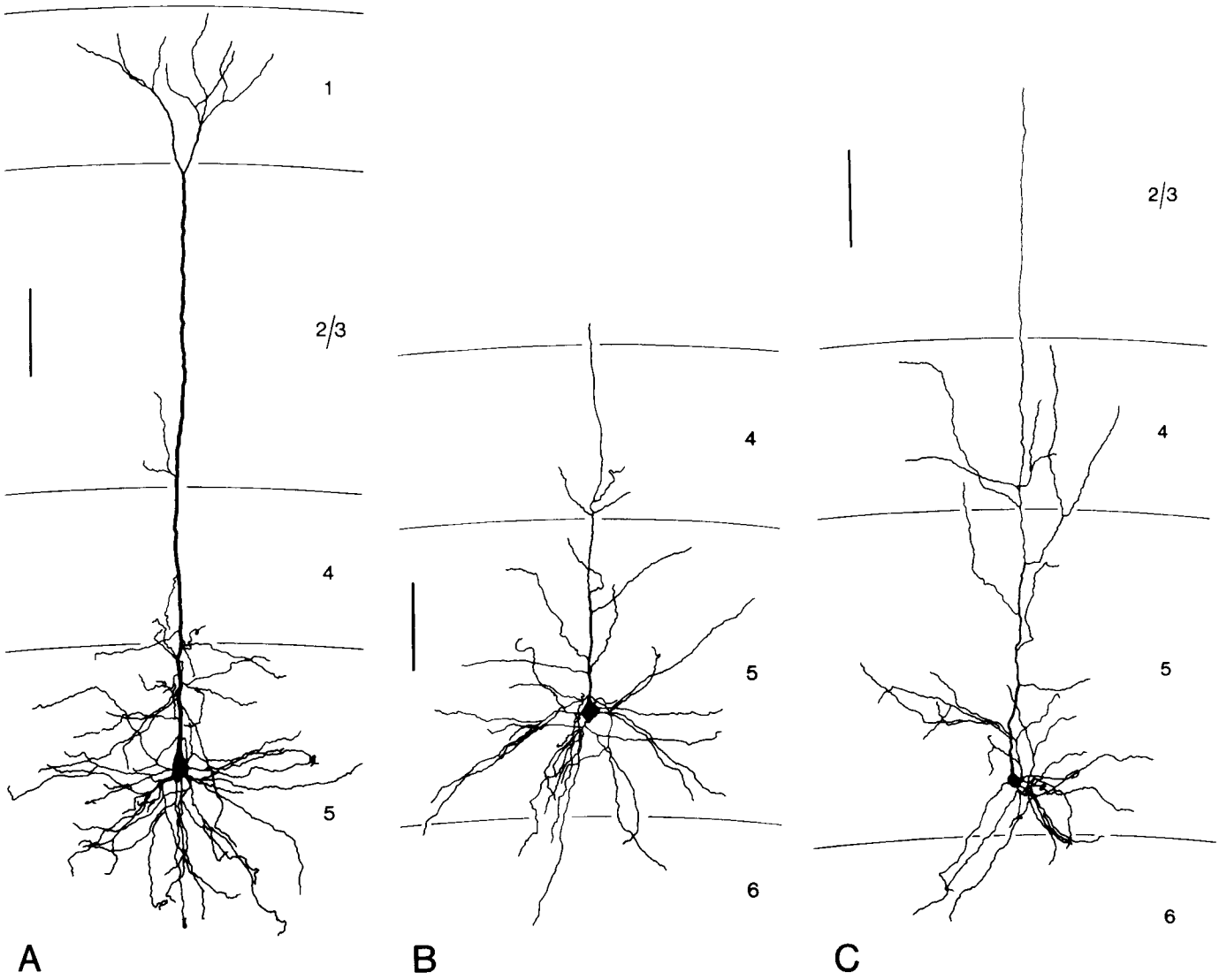

Figure 2. Thick (*A*) and slender (*B* and *C*) layer 5 cells. Scale bars, 100 μm. *A*, Thick cell from the upper part of layer 5. The thick apical trunk gives off numerous oblique branches close to the soma, then traverses layer 2/3 with little branching or tapering to form a terminal arbor in layer 1. *B*, Slender cell from the lower half of layer 5. The apical trunk tapers to a fine process which terminates, without forming a terminal arbor, near the top of layer 4. *C*, Slender cell from the lower part of layer 5, of different morphology than *B*. The apical trunk tapers as it ascends and gives off oblique branches, some of which arise at a considerable distance from the soma and ramify in layer 4. These branches were not considered to represent a terminal arbor because a fine continuation of the apical trunk extended beyond them and terminated in the upper part of layer 2/3.

Table 1. Soma size and numbers of dendritic tips

	L2/3 (n = 18)	Slender L5 (n = 10)	Thick L5 (n = 11)
Soma height (μm)	18.4 ± 2.2	19.8 ± 3.0	24.5 ± 4.9[a]
Soma width (μm)	14.7 ± 1.8[a]	19.3 ± 3.9	18.1 ± 3.3
Height/width	1.26 ± 0.14	1.05 ± 0.20[a]	1.36 ± 0.19
Basal tips	29.2 ± 7.5	25.8 ± 5.4	34.6 ± 5.7[a]
Oblique tips	11.9 ± 6.1	13.7 ± 7.0	24.6 ± 3.1[a]
All tips	49.8 ± 12.7[b]	40.5 ± 6.0[b]	65.2 ± 6.8[b]

Here and in Table 2, data are presented as mean ± SD. Significant differences between cell groups (at $p \leq 0.05$) are indicated as follows:

[a] = difference between this group and both other groups.

[b] = difference between all 3 groups.

[c] = difference between these 2 groups.

for thick L5 cells were significantly greater than those for L2/3 or slender L5 cells. Somal widths of the thick L5 cells were significantly greater than L2/3, but were similar to slender L5 cells. The height/width ratios confirm that both L2/3 and thick L5 somata were significantly more elongated vertically than those of the slender L5 cells.

Numbers of dendritic segments

The dendritic system of a neocortical pyramidal cell can be divided into basal and apical components. The apical component can be further subdivided into the trunk and the oblique and the terminal dendritic arbors. A count of the number of dendritic segments or tips in each of these categories represents an index of the dendritic geometry of a cell which may be used to highlight differences between cell classes (Table 1). Such an index should be relatively free from measurement error. One

problem that was encountered, however, was the probable loss of parts of the terminal arbor of thick L5 cells during slice preparation. This loss would not affect any of the conclusions described here (see Discussion).

Basal tips. The number of basal tips per cell varied between 19 and 49. L2/3 cells had similar numbers to slender L5 cells, but both classes had significantly fewer than thick L5 cells. The thick L5 cells also showed less variability in this measure than slender L5 or L2/3 cells.

Oblique tips. The number of oblique tips per cell was more variable than for basals, ranging between 4 and 33, but the pattern of differences between the cell classes was the same. L2/3 cells had similar numbers to slender L5 cells, and both had significantly fewer than thick L5 cells. A striking feature was the considerably lower variability seen among the thick L5 cells than the other cell classes.

Total numbers of tips. When all the dendritic tips were considered together, the slender L5 cells were found to have significantly fewer tips than the L2/3 cells, which in turn had significantly fewer than the thick L5 cells (in spite of the probable loss of some of the terminal arbor of the latter).

Mean path lengths

Basals. The mean path length of the basal dendrites of each cell, measured from soma to tip, was calculated and pooled by cell class (Table 2). It was found that slender L5 cells had significantly longer basal dendrites than either L2/3 or thick L5 cells. The thick L5 class showed less variation between cells than the other classes.

Obliques. The path length of each oblique dendrite, from its point of departure from the apical trunk to its tip, was measured. Mean values were calculated for each cell and pooled by cell classes (Table 2). The path lengths of the oblique dendrites, measured in this way, were generally shorter than for the basals, but the pattern of differences between cell classes was the same as for the basals. Slender L5 cells had significantly longer oblique dendrites than either thick L5 cells or L2/3 cells, which were not significantly different from each other. The thick L5 cells showed much less between-cell variation than either of the other cell classes. Three slender L5 cells were unusual in having mean path lengths of their oblique dendrites longer than their basals.

Combined dendritic length

The lengths of all the individual dendritic segments were added together to derive the combined dendritic length for each neuron (Table 2). The L2/3 and slender L5 cells were of similar combined dendritic length, and the thick L5 cells were significantly larger. When only the basal dendrites were considered, it was found that the slender and thick L5 cells were not significantly different from each other, but both were significantly larger than the L2/3 cells. For L2/3 and slender L5 cells, the combined length of the basals exceeded that of the apicals, but they were almost equal for the thick L5 class.

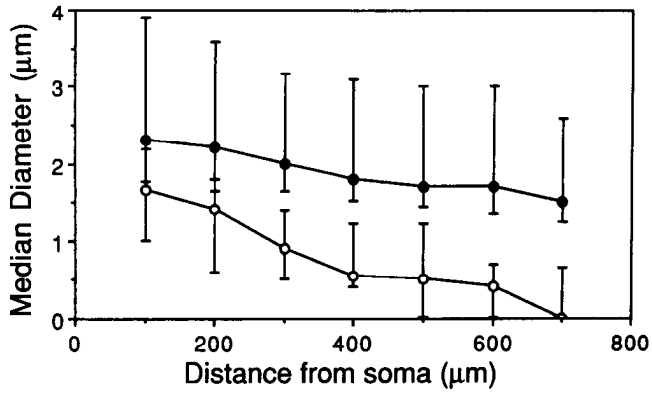

Figure 3. Apical trunk diameters of layer 5 cells. The diameters of the apical trunks of thick (*filled circles*) and slender (*open circles*) cells were measured at various distances from the soma. *Points* indicate the median and *error bars* the range of values recorded for each class.

Dendritic shaft membrane area

By making use of the diameter measurements as well as dendritic lengths, it is possible to estimate the combined membrane area of the dendritic shafts of a given cell. This measure ignores the membrane area contributed by the numerous dendritic spines, which will be considered in a future publication. The differences between the cell classes were found to be more marked than for combined dendritic length, although the pattern was similar (Table 2). Values obtained for L2/3 cells were significantly smaller than for slender L5 cells, which in turn were significantly smaller than thick L5 cells. The cell classes also differed in the way in which the dendritic shaft membrane area was distributed between the basal, oblique, and apical trunk dendrites. The shaft area of the basal dendrites of L2/3 cells was significantly smaller than for the thick and slender L5 cells, which were not significantly different from one another. If the apical oblique dendrites were considered separately, it was found that the slender L5

Table 2. Dendritic lengths and areas

	L2/3	Slender L5	Thick L5
Mean path lengths (μm)			
Basal dendrites	144 ± 25	184 ± 38[a]	157 ± 19
Oblique dendrites	116 ± 22	160 ± 33[a]	127 ± 15
Combined dendritic lengths (mm)			
Basal dendrites	3.33 ± 0.92[c]	4.06 ± 1.19	4.51 ± 0.80[c]
All dendrites	5.86 ± 1.51	6.60 ± 1.21	8.95 ± 1.06[a]
Dendritic shaft membrane area (μm²)			
Basal dendrites	7521 ± 2186[a]	10,360 ± 3011	12,277 ± 4003
Oblique dendrites	2366 ± 1246[b]	4093 ± 1535[b]	7119 ± 1861[b]
All dendrites	12,750 ± 3350[b]	16,231 ± 3351[b]	26,200 ± 6687[b]

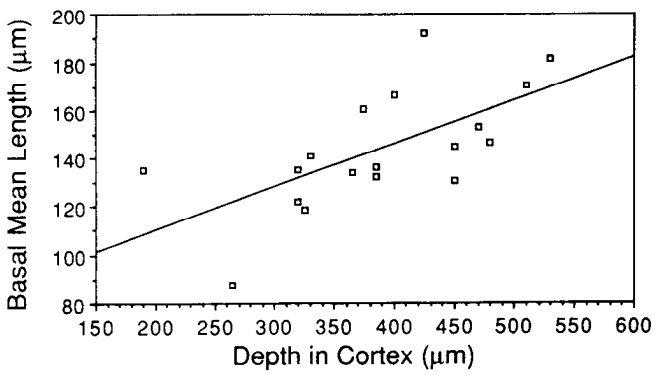

Figure 4. Relationship between the length of basal dendrites and the depth of the soma in the cortex for layer 2/3 cells. The mean path length of the basal dendrites of each cell was found to be positively correlated with the cortical depth of the soma (Kendall's tau = 0.52, $p < 0.01$).

cells had significantly larger oblique arbors than the L2/3 cells, but both were significantly smaller than the thick L5 cells. For thick L5 cells, the basal arbor comprised almost half the total shaft area, and for L2/3 and slender L5 cells the proportion was even higher, approximately 60%. The contribution to the total shaft area made by the oblique dendrites was 19% for layer 2/3 cells, 25% for slender L5 cells, and 27% for the thick L5 cells. The loss of part of the terminal arbors of some of the thick L5 cells is not thought to have substantially affected these findings.

Correlations with depth in layer 2/3

Several quantitative features of somadendritic morphology of L2/3 cells were found to be correlated with the depth of the soma in the cortex. The mean path lengths of the basal dendrites were positively correlated with depth in the cortex (Fig. 4). The number of dendritic tips in the terminal arbor was inversely correlated with depth (Kendall's tau = 0.53, $p < 0.001$), as was the proportion of the total dendritic shaft area contributed jointly by the basal and oblique dendrites (Kendall's tau = 0.62, $p < 0.001$; not illustrated). These correlations support the notion of there being a continuum of dendritic morphologies spanning the depth of layer 2/3.

Discussion

An important question addressed in this study is whether the different pyramidal morphologies encountered within and between cortical layers form a continuum, or whether they reflect the presence of several distinctive cell types, possibly tailored for particular functions. Our sample of cells from layer 2/3 showed variation in both size and form, but appeared to share a common basic structure. Several features of their morphology varied progressively with the depth of the soma in the layer, without any obvious discontinuities. We could also find no evidence of a sharp distinction between layers 2 and 3. Only one L2/3 cell differed from the others in a possibly fundamental way. This was the cell located at the upper boundary of the layer which did not have an apical trunk, but had 2 ascending dendrites arising from the soma. Cells of this appearance have been described from the same laminar position in the visual and motor cortices of the cat and rat (O'Leary, 1941; Parnavelas et al., 1983; Peters, 1985; Ghosh et al., 1988) and have been termed modified superficial pyramids. Peters (1985) argued that these cells represented the extreme of the continuum of pyramidal cell forms in layer 2/3. We therefore concluded that our sample of layer 2/3 cells displayed a range of forms changing with depth, but represented a single cell class.

Within layer 5, however, it was possible to subdivide the sample into 2 major classes, which we termed thick and slender cells. The thick cells varied in overall size, but showed a striking constancy of dendritic pattern, with lower between-cell variation for a number of features than the other classes. In our opinion they represented a homogeneous cell class. The slender cells were all readily distinguishable from the thick, but showed some heterogeneity of dendritic pattern and possibly contained examples of more than one cell type. An obvious possible subgroup might be the 3 cells whose apical dendrites terminated near the top of layer 4. We also recovered a single cell with twin apical trunks, which had some features in common with both the other classes and, in our opinion, could not be readily included with either.

It is possible that, with improved staining and visualization techniques, small terminal arbors might have been seen on the fine terminal processes of the apical trunks of some of the slender L5 cells. Given the small diameters of these processes, it is likely that any such arbor would be small, clearly distinguishable from those of thick cells, and would make little difference to the quantitative measures we obtained. However, the application of alternative tracers coupled with electron microscopy might be a useful extension to this study.

Atypically oriented pyramidal cells of several types have been reported from a number of mammalian cortices, including the visual cortex of the rat (Parnavelas et al., 1983; Miller, 1988). The only examples of atypically oriented cells in our sample were cells with displaced cell bodies (Miller, 1988). The functional significance of this feature is not known, but it is interesting that it was not uniformly distributed among the different cell classes. Its occurrence was restricted to those slender L5 cells with apical dendrites extending into layer 2/3, and 5 out of 7 of these cells had displaced cell bodies.

Previous classifications of rat layer 5 cells

Several previous studies have indicated the existence of more than one type of pyramidal neuron in layer 5 of the visual cortex of the rat. Peters and coworkers (Peters, 1985; Peters and Kara, 1985, 1987; Peters et al., 1985) distinguished 2 types, which they termed large- and medium-sized pyramids. Their classification was based on features such as soma size and apical dendrite diameter which could be distinguished in thin-sectioned material. Their large cells had thick apical trunks which arose from a gradual tapering of the soma, while the medium cells had thinner apical trunks which arose more abruptly. There would thus appear to be a correspondence between their large and medium categories and our thick and slender classes. Their measurements of apical trunk diameters in the 2 classes (Peters and Kara, 1987) are in good agreement with our own. We have refrained from using their terminology only because of the substantial overlap in soma size and measures of dendritic extent between the 2 classes in our sample. Features such as apical dendritic branching pattern are, in our opinion, of equal or greater importance than size in separating the cell types.

Other recent studies have classified layer 5 cells on the basis of their morphology and projection targets. Cells projecting to the superior colliculus and pons have been shown to have thick apical dendrites with prominent terminal arbors in layer 1 (Schofield et al., 1987; Hallman et al., 1988; Hübener and Bolz,

1988) and the morphology of these cells is similar in all respects to our thick L5 class. Corticotectal cells of similar appearance have also been described from hamster visual cortex (Klein et al., 1986). A second type of cell, with different somadendritic morphology, which projects to the contralateral cortex has been identified by retrograde labeling. These cells have more rounded somata and fewer primary basal dendrites than corticotectal cells, and their apical dendrites are shorter, never reaching layer 1 (Hallman et al., 1988; Hübener and Bolz, 1988). These callosally projecting cells have many features in common with our slender L5 cells.

It has been suggested that layer 5 of the rat visual cortex can be divided into 2 sublaminae with different cell populations (Ryugo et al, 1975; Connor, 1982). Our thick cells were restricted to the upper part of the layer, while slender cells were distributed throughout. This pattern is consistent with the partial laminar segregation of corticotectal and callosal cells as revealed by retrograde labeling (Hallman et al., 1988; Hübener and Bolz, 1988).

Comparison with previous quantitative studies

There have been a number of quantitative light microscopic studies of pyramidal cells from the rat visual cortex using Golgi-based methods. Our estimates of dendritic lengths are generally greater than those of previous studies. The mean lengths of basal and oblique dendrites for the L2/3 cells of our sample are some 40% greater than those found by West and Kemper (1976). Our estimates of combined dendritic length are greater by a factor of approximately 3 than those obtained for the basal trees of layer 3 and 5 pyramids by Juraska (1982, 1984) or for the large layer 5 pyramidal cells of rat sensorimotor cortex obtained by Petit et al. (1988). Comparisons such as these give us confidence that our procedures can lead to a more complete visualization of the extent of dendritic arbors than is routinely possible by Golgi-based methods.

Much of the difference between our values and those of previous studies may be due to our ability to reconstruct cells reliably across tissue section boundaries, and our attempt to correct for 2-dimensional projection errors. An example of a similar situation may be found in the hippocampal literature. Desmond and Levy (1982) measured combined dendritic length for dentate granule cells. They used Golgi preparations, but corrected for projection errors and used a novel probabilistic method to correct for the portions of the dendritic arbors lost during sectioning. Their estimates of combined dendritic length exceeded those of previous studies, which had not made such corrections, by up to an order of magnitude. Desmond and Levy (1982) estimated that the small deviations in the course of dendrites, orthogonal to the plane of focus, which they termed "wiggles," could lead to dendritic lengths being underestimated by up to 20%. We did not correct for wiggles in the present study, so our values for individual and combined dendritic lengths may still be underestimated.

An interesting and unexpected finding from this study has been the relative importance of the basal and oblique dendrites. We found that the basal and apical oblique dendrites together often contribute over 70% of the total dendritic shaft membrane area of the cell. As pointed out by Ghosh and coworkers (Ghosh and Porter, 1988; Ghosh et al., 1988), for many pyramidal cells most of the oblique dendrites arise close to the soma. Thus, the basal and proximal oblique dendrites together sample a roughly spherical volume of cortex centered about the soma, while the apical trunk, terminal arbor, and distal obliques sample a rather different cortical volume, perhaps extending into different cortical layers. The basal and proximal oblique dendrites may represent a functionally distinct, and quantitatively highly important, subdivision of the overall dendritic system.

In conclusion, it was possible to divide the sample of neurons into 3 classes using qualitative features of their somadendritic morphology, and this classification was supported by a range of quantitative measures. The L2/3 cells were all placed in a single class and the L5 cells were divided into 2 classes. This classification of L5 cells appeared consistent with previous classifications based on their projection targets. The intrinsic electrophysiological properties of the cell classes established in this paper will be described and compared in the following paper (Mason and Larkman, 1990).

Appendix

The use of intracellular HRP injection in slices maintained *in vitro* offers a number of advantages. Stable intracellular impalements are relatively easy to obtain *in vitro*, so electrophysiological recordings of high quality and morphological data can be obtained from the same cell. Single neurons can be stained in isolation and the probability of successful recovery is high (Mason et al., 1988). Because the single neurons can easily be reconstructed across tissue sections, the sections can be made relatively thin (60 μm in this case), and thus can be viewed using high-power light microscope objectives. However, this approach is not without its disadvantages, some of which are considered below:

1. Maintenance in vitro. Tissue is maintained for a period *in vitro* prior to fixation, during which its condition may deteriorate. However, we have previously shown that the central portion of the slice can remain in good condition for the duration of our experiments (Larkman et al., 1988). We therefore took care to inject neurons only from the central regions of slices, and all the cells displayed apparently normal electrical activity prior to injection. None of the cells selected for morphological analysis showed signs of degeneration likely to have been caused by the period of maintenance *in vitro*.

2. Loss of dendrites during slice preparation. The nominal 400-μm thickness of the slices makes it possible that a portion of the dendritic arbor of some cells has been amputated during slice preparation. For most cells, this did not appear to be a serious problem. We injected only cells whose somata lay in the central region of the slice, and during reconstruction it was observed that the vast majority of dendrites terminated within the slice. A small number of dendrites from L2/3 and slender L5 cells were seen to reach the surface of the slice, but their number never exceeded 5 for any cell that was accepted for analysis. The estimated resultant loss of dendritic length never exceeded 5% of the cell's total, which is within measurement error. This is in agreement with the finding that 93.5% of the basal dendrites of pyramidal neurons with cell bodies in the central regions of 350-μm-thick sections of rabbit auditory cortex terminate within the section (McMullen and Glaser, 1988). It seems likely that the problem of dendritic loss will affect most severely those cells with the longest dendrites. Thus, the effect will be to minimize the differences in dendritic lengths between cell classes reported here.

A more serious problem arose with the terminal arbors of the thick L5 cells. Although the somata of these cells were always located near the center of the thickness of the slice, unless the apical dendrite lay precisely in the plane of the slice, the point of origin of the terminal arbor could be closer to one surface. Given the widespread terminal arbors seen in some of these cells, it seems likely that a proportion of the arbor will have been lost in some cases. Six out of our sample of 11 thick cells were obviously affected in this way, and others may have been to a lesser extent. Thus, the size of the terminal arbors of thick L5 cells will have been underestimated in this study. Thick L5 cells were found to have a greater number of apical dendritic tips than the other cell types; presumably this difference would have been more marked had some of their terminal arbors not suffered partial amputation. Although some loss of dendrites inevitably has occurred during slice preparation, it is not thought to have influenced any of the conclusions reached in the present study.

3. Electrode sampling. There is no reason to believe that intracellular microelectrodes sample neurons randomly. It is widely held that cells with larger somata will be impaled with a higher probability (Ghosh et

al., 1988). Additionally, there was a tendency during our experiments to concentrate the electrode tracks in the central regions of the relevant cortical layers to avoid uncertainty over the precise location of laminar boundaries. Thus the numbers of cells in the various classes in our sample may not accurately reflect their relative abundance in cortex.

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