Morphology and intracortical projections of functionally characterised neurones in the cat visual cortex

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The neuronal structure and connectivity underlying receptive field organisation of cells in the cat visual cortex have been investigated. Intracellular recordings were made using a micropipette filled with a histochemical marker, which was injected into the cells after their receptive fields had been characterised. This allowed visualisation of the dendritic and axonal arborisations of functionally identified neurones

CONSIDERABLE insight into the stages of visual information processing has been obtained from analysis of single cell responses to patterned light stimulation. This approach revealed that cells in the visual cortex respond optimally to specific stimuli, such as line segments of given orientation and length[1,2], and that neurones differ in their specificity. Cortical neurones also show great morphological diversity[3-7], suggesting that there may be a correlation between the function of a cell and its structure. Another feature of cortical organisation is that cells are segregated into layers, and in each layer the cells have distinct size, shape and packing density. This lamination has long been thought to be important in cortical function[8-10]. The layers differ further in the receptive field properties of the cells within them[2,11-13], in the inputs that they receive[14-17] and in the sites to which their cells project[18-22]. This information was derived from extracellular recording and anotomical tracing, but now intracellular marking techniques, first used in the visual cortex by Kelly and Van Essen[23], can extend these findings by showing the detailed intracortical wiring of cells with known response properties.

In this study we have been able to trace the intracortical projections of functionally identified neurones in different layers by intracellular injection of the enzyme horseradish peroxidase (HRP)[24,25]. Comparison of the receptive field properties of marked cells with those of cells in the layers to which they project provides evidence for the manner in which receptive fields are constructed.
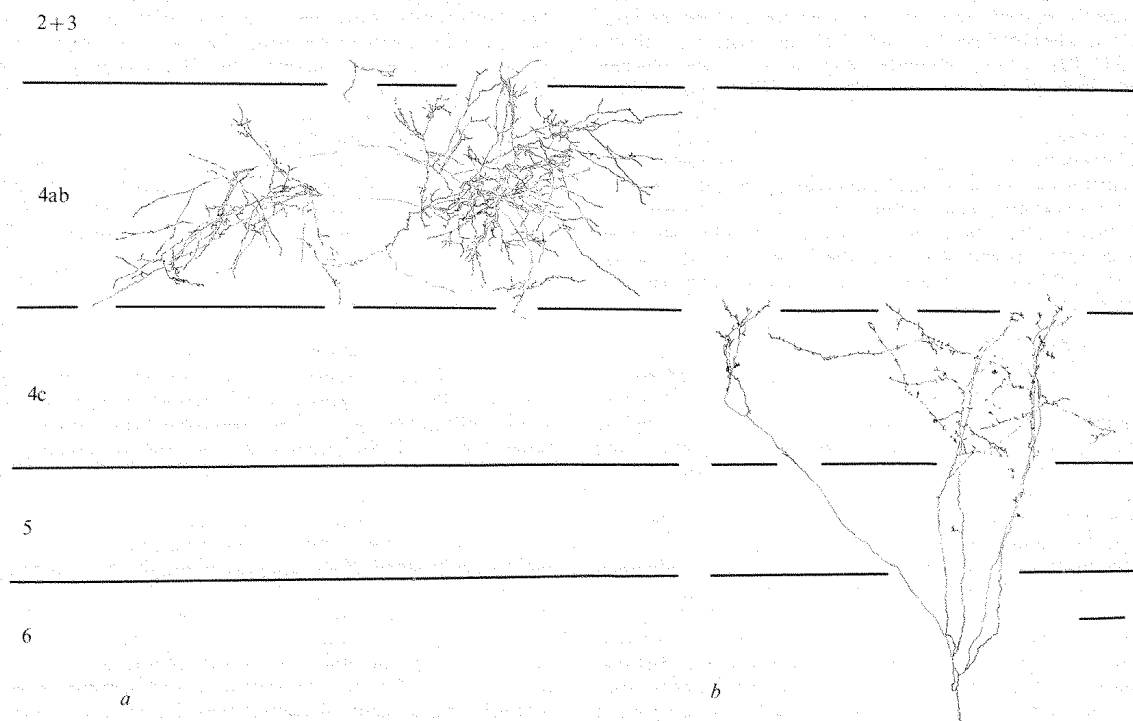
Fig. 1. Two afferents from the lateral geniculate nucleus, injected within the cortex. *a*, An off-centre Y-cell (centre size 1.5°, located 5° from the area centralis), and ramified entirely within layer 4ab. *b*, An off-centre X-cell (centre size 1°, located 3° from the area centralis), which ramified entirely within layer 4c. Scale bar, 100 μm.

Table 1   Numbers and laminar positions of injected cells

Layer	No. of cells
2 + 3	16
4ab	4
4c	2
5	19
6	11
Afferents	9
Total	61

Functional characterisation and labelling of visual cortical neurones

Recordings were made in cats maintained on sodium thiopental anaesthesia, paralysed with succinylcholine and artificially respirated. The animals' electrocardiogram, electroencephalogram, temperature and expired CO_2 concentration were monitored. A small hole was drilled in the skull above the visual cortex, and the dura and pia were opened. Half-micrometre bevelled-tip electrodes were filled with a solution of 4% HRP (Boehringer–Mannheim, grade I) in 0.2 M KAc, pH 7.6. After penetration of the brain surface with the electrode, the hole in the skull was filled with agar to reduce pulsations. The electrodes were advanced through the brain with a stepping microdrive (Transvertex) until a cell or process was penetrated, as indicated by a sudden change in the resting potential, the appearance of large action potentials and often synaptic activity. The receptive field properties of the cells were determined using either a hand-held projector or an optical bench.

The cells were injected with HRP either by pulses of pressure ranging from 0.5 to 5 atm or by pulses of positive current ranging from 1 to 2 nA in a 4 ms-on/4 ms-off duty cycle. At the end of the experiment each animal was perfused with a short buffer rinse followed by a long perfusion with 2% glutaraldehyde in the same buffer. After the brain was blocked, 150-μm coronal sections were cut on a Vibratome (Oxford Instruments), and then treated with a combination of the 3,3'-diaminobenzidine reaction at acid pH[26] and cobalt intensification[27]. The injected cells were reconstructed from serial sections by using a microscope equipped with a drawing tube. Subsequently, the sections were counterstained with cresyl violet, and the laminar position of cells and their processes were determined using the criteria of Otsuka and Hassler[28]. Table 1 lists the cells in each layer that we have injected to date.

Cortical afferents

Different morphological and physiological classes of retinal ganglion and geniculate cells are known to exist[29–32]. Chief among these are the X- and Y-cells. These are defined by the linearity of spatial summation within their receptive fields[29], and also have been found to differ in their firing properties and axon conduction velocities[30,33,34]. The dorsal geniculate laminae, which contain a mixture of X- and Y-cells[35], project to layers 4 and 6 in the visual cortex[16,17].

In this study we were able to penetrate and inject individual axons coming from the lateral geniculate nucleus either before or after they enter the cortex. We used the criteria of spatial summation to classify X- and Y-afferents[29,36]. A reconstruction of each type is shown in Fig. 1. The Y-afferent (Fig. 1a) had off-centre/on-surround organisation, showed nonlinear summation, had a relatively large field centre (1.5°) and gave a transient response to a stationary flashing spot of light. It ended in layer 4ab in a rich arbor distributed in two patches separated by a terminal-free gap. The patches presumably correspond to ocular dominance columns driven by one eye, and the intervening gap to the column driven by the opposite eye[15,37] Some of the injected Y-afferents also sent collaterals to the upper half of layer 6.

In contrast to the afferents showing nonlinear summation, the X-afferent arborised entirely within layer 4c (Fig. 1b). No afferents were found to arborise in both sublayers of layer 4. This demonstrates, in agreement with Ferster and Levay[38], that the dorsal geniculate layers, which contain a mixture of at least two principal cell types, keep the input from the two types segregated on their arrival in the cortex.

Layer 4 cells

Cells in layer 4 lie in the terminal field of the geniculate afferents, and consequently represent the first level of processing in the cortex. Extracellular recordings show that the overwhelming majority of the cells in layer 4 have simple receptive fields[1,11,23], as defined by Hubel and T.N.W.[1]. In addition to their specificity for stimulus orientation, simple cells can show a reduction in response to slits longer than the receptive field[11,39,40]. This property, known as end-inhibition, makes cells optimally responsive to orientated lines of a defined length. Figure 2a shows an example of a simple cell in layer 4ab. It was a spiny stellate cell; the axon branched several times soon after leaving the soma, with a number of collaterals innervating in layer 4ab and then giving off a rich terminal arborisation in layer 2 + 3. The axon proceeded out of the cortex into the white matter, sending a few collaterals into the lower layers in its downward course. This general projection pattern was seen for all injected 4ab spiny cells and has also been observed with Golgi stains[3–6]. The horizontal extent of the axonal arborisation was much larger than that of the dendritic arborisation. This divergence could produce a further mixing in the input from the two eyes onto layer 2 + 3 cells, and could account for the higher proportion of binocularly driven cells found in that layer than in layer 4 (refs 1, 11).

Only two cells have been injected in layer 4c, one a small spiny stellate cell and the other a smooth stellate cell (Fig. 2b). They had very similar receptive field properties; both had simple receptive fields with on-centres, which were much smaller than those of the layer 4ab simple cells. The smooth stellate cell's axon ramified extensively throughout layer 4. The axon of the spiny stellate cell gave off many collaterals, some remaining within layer 4c and others extending to layer 4ab. Although the cell's axon was restricted to layer 4 (which is consistent with Golgi findings[5]), the existence of complex cells with very small receptive fields in layer 2 + 3 (refs 1, 11) leads us to expect that there should be other layer 4c cells with projections to that layer.

Layer 2 + 3 cells

The predominant intracortical projection from layer 4 seems to be to layer 2 + 3, which then represents the second level of cortical processing. Layer 2 + 3 almost exclusively contains complex cells[1,11]. They are orientation selective, but differ from simple cells in having uniform receptive fields not divisible into separate on- and off-subregions and in responding continuously as a slit of light is moved across their fields[1,11,41]. Like simple cells, they can be directional and end-inhibited. For complex cells, specificity for orientation and length is maintained, but compared with simple cells they have gained some freedom in the precise position of the stimulus along the movement axis[1].

A complex cell with a small receptive field (1° × 1°), showing no end-inhibition, is shown in Fig. 3. It was a pyramidal cell located in the upper part of layer 2 + 3. The basal dendrites ramified close to the soma and the apical dendrite extended into the lower part of layer 1. Its axon branched richly in its layer of origin, both in a region adjacent to the cell's dendritic field and in a region rather more distant, perhaps to a column of cells with the same orientation preference. The axon collaterals also extended into layer 1. The descending axon projected extensively within layer 5; this was a common feature of pyramidal cells in this layer, in agreement with degeneration[42–44] and Golgi[4,5,45] studies. The axon proceeded out of the cortex, presumably to innervate another cortical area or areas[21].

1

2+3

4ab

4c

5

6

a

1

2+3

4ab

4c

5

6

b
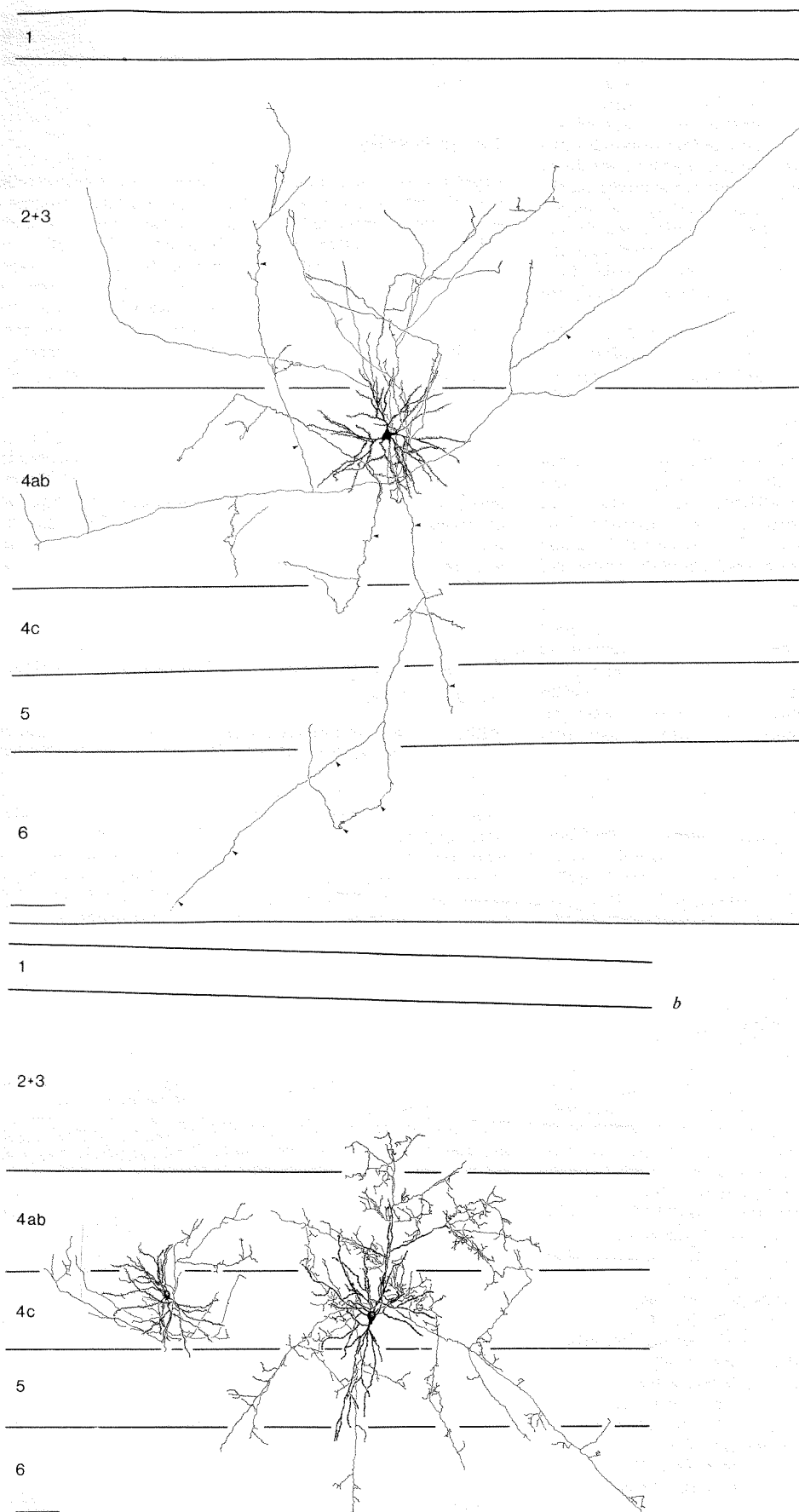
Fig. 2 *a*, A spiny stellate cell in layer 4ab having a simple receptive field with an on-centre and off-flanks, and showing no end-inhibition. The field size was $3° \times 4°$, and it was located 10° from the area centralis. The arrows indicate the positions of nodes of Ranvier. *b*, A spiny stellate cell (left) and a smooth stellate cell (right) in layer 4c. Both had simple receptive fields with on-centre and off-flanks, the spiny stellate cell showing no end-inhibition and the smooth stellate cell showing 50% end-inhibition. The size of the central on-portion of the field for both cells was $1° \times \frac{1}{2}°$, and both were located 2° from the area centralis. Because the plane of section was not perpendicular to the layers, this reconstruction makes it seem as if several sets of collaterals of the smooth stellate cell extend into layers 5 and 6, whereas in fact they remain within 4c. Scale bar, 100 μm.

Layer 5 cells

Cells in layer 5 have complex receptive fields like those in layer 2+3, but differ most notably in their large field sizes. Previous studies[11,20] have shown that within layer 5 two cell types can be distinguished: one that shows summation for increased slit length (the standard complex cell) and one that responds optimally to a small moving slit of light placed anywhere within its relatively large field, showing no summation for increased slit length (the special complex cell). Consequently, the special complex cell maintains specificity for orientation, length and direction of stimulus movement, but gains some freedom in the precise position of the stimulus along the orientation axis. Like simple and standard complex cells, the special complex cell can exhibit end-inhibition[11].

Figure 4 shows a standard complex cell in layer 5. A surprising finding was that the cell's axon sent an extensive projection to layer 6, passing 6–8 mm down the medial bank, still remaining within area 17. The cell's receptive field was 2¾°×1¾°, yet its axon spanned an area of cortex representing up to 15° of visual field. Thus, it reaches areas that deal with parts of the field of view far outside the cell's receptive field. This projection has not been seen with Golgi stains, possibly because the axon becomes myelinated soon after leaving the cell body, and would therefore not impregnate with the Golgi technique. The distribution of the axonal field as viewed from the cortical surface was long and narrow. One might expect that the overall axis of distribution of the axon would be related to the orientation axis of the cell's receptive field. The axonal distribution and orientation of this cell were consistent with this view. The apical dendrite extended up into layer 1, branching repeatedly there, and several of its processes passed just underneath the pia. The cell had a dense dendritic arbor near its cell body, with a number of processes leaving the base of the apical dendrite at the layer 4/5 border, and a set of basal dendrites extending down into the upper part of layer 6.

We have also injected special complex cells in layer 5. Their axons seemed not to project as richly to layer 6 as those of the standard complex cells, and set a large trunk out of the cortex, presumably to innervate the superior colliculus[20,21].

Layer 6 cells

Layer 6 is of special interest as it receives a direct projection from the lateral geniculate nucleus[11] and contains a mixture of simple and complex cells[1,11,23]. Its cells have unique receptive field properties, often requiring long slits for activation and showing summation with slit length up to very large values (16°)[11]. Figure 5 shows an example of a layer 6 simple cell. The basal dendrite of the cell branched in the upper part of the layer; precisely the part in which the collaterals of geniculate axons end. Its axon projected mainly to layer 4ab, in the vicinity of its apical dendrite, which branched extensively and ended in 4ab. Other cells, both pyramidal and smooth stellate, had axonal fields that lay almost exclusively within layer 6.

Although the simple cells of layer 6 were restricted to the upper half of the layer, we found complex cells throughout the layer. The apical dendrites of these cells branched at different levels within the cortex, which is reminiscent of the pattern observed in the monkey by Lund and Boothe[45]. Like the cell in Fig. 5, they often sent collaterals primarily to layer 4, and these were restricted to either 4ab or 4c. The injected smooth stellate cell had a complex receptive field.

Intracortical pathways and receptive field construction

Although we have not yet injected all the morphological cell types found in the cortex[4], some patterns of projection have
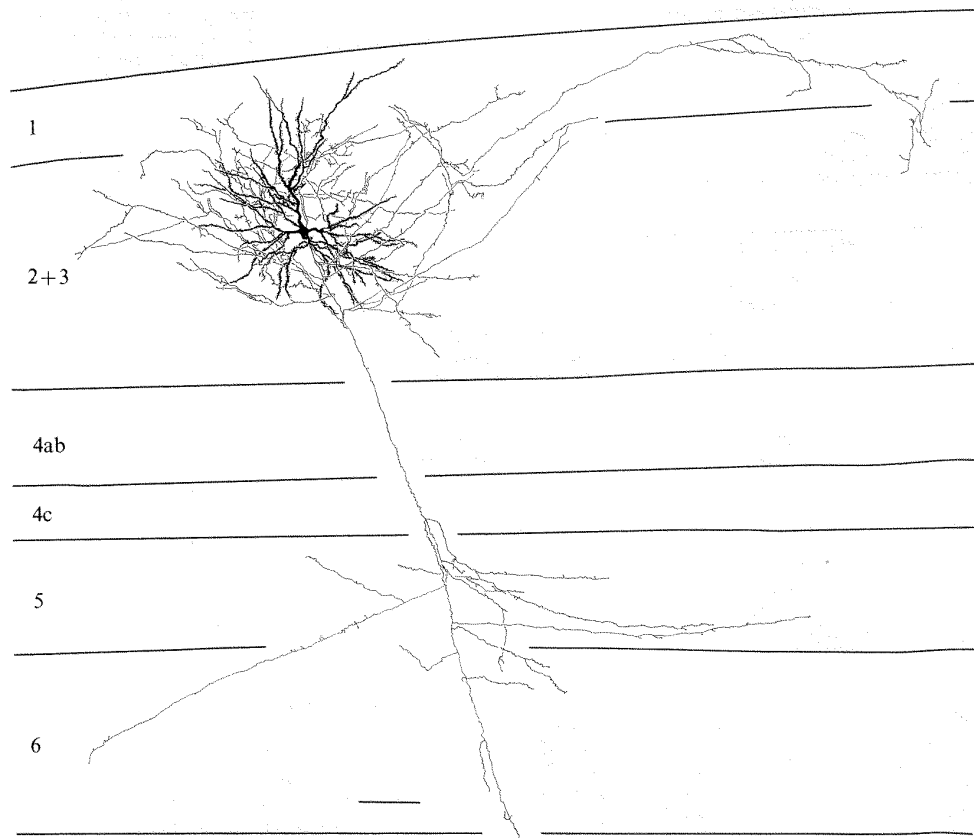
Fig. 3 A pyramidal cell in the upper part of layer 2+3, with a complex receptive field showing no end-inhibition. The field size was 1°×1°, and it was located 4° from the area centralis. Scale bar, 100 μm.
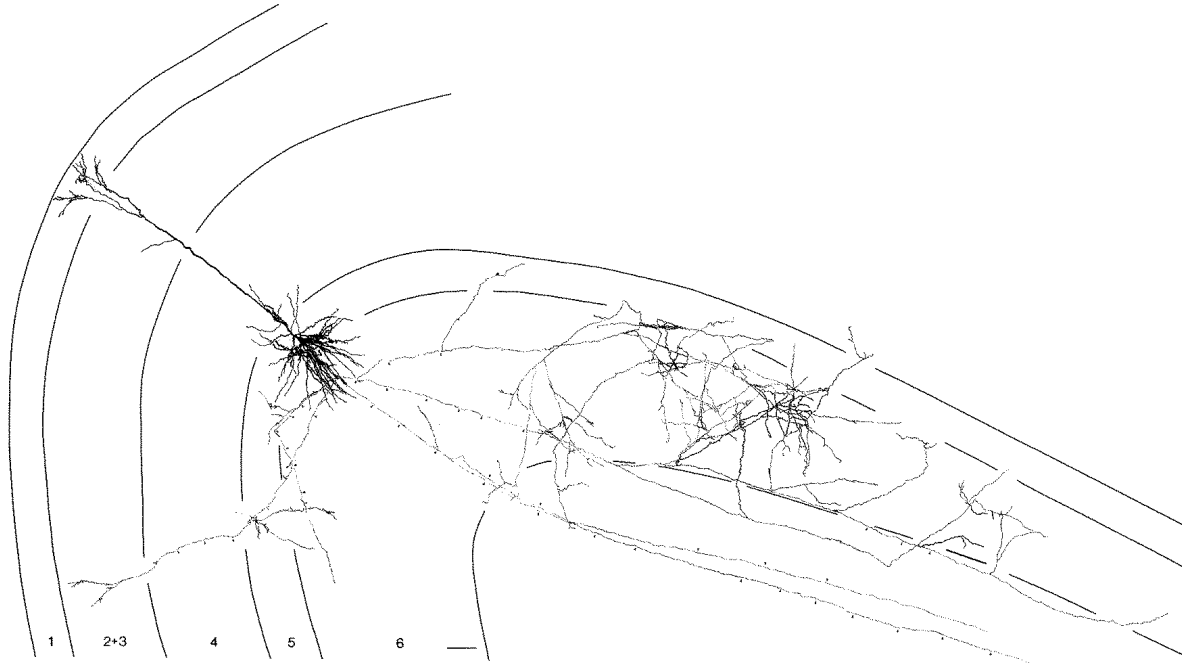
Fig. 4 A pyramidal cell in layer 5, with a standard complex receptive field showing no end-inhibition. The field size was $2\frac{3}{4}° \times 1\frac{3}{4}°$, and it was located on the area centralis. The branches of the apical dendrite within layer 1 all run quite close to the pia, but this is not apparent due to the plane of the section relative to the cortical layers. Scale bar, 100 μm.

emerged from our present catalogue of injected cells. The predominant stages of information processing in the cortex seem to be as follows: the major geniculate input to the cortex arrives in layer 4, with the X-input and the Y-input arriving separately in layers 4c and 4ab. Both inputs also project to the upper half of layer 6. From layer 4 the sequence of processing continues to the upper layers, from the upper layers to layer 5, and from layer 5 to layer 6. Layer 6 then sends output back into layer 4. The cortex is tapped for output to other regions at several stages: cortical areas from layer 2 + 3, superior colliculus from layer 5, and lateral geniculate nucleus from layer 6. Also, the spread seen in the horizontal axonal projections (Fig. 4) extends far beyond that expected from previous studies using the Golgi technique[3-6].

This pattern enabled us to form hypotheses as to the manner in which receptive fields are constructed within the cortex. As originally suggested by Hubel and T.N.W.[46], simple receptive fields are constructed from the fields of lateral geniculate neurones, as simple cells are the predominant class in layer 4, the major geniculate afferent zone. The correspondence between simple receptive field type and geniculate input is also seen for the cells that lie in the upper part of layer 6. Although they share the same general receptive field type, cells in layer 4c have smaller fields than those in 4ab. This difference is consistent with the two features of the geniculate input to layer 4—the X-afferents have smaller receptive fields than the Y-afferents, and the 4ab afferents have a much wider terminal arborisation than the 4c afferents[38]. Although the receptive fields of the injected 4c cells were quite similar, spiny cells are thought to be excitatory and smooth cells inhibitory. These presumptions are based on both their synaptic morphology and transmitter neuro-chemistry[47-49].

The superficial layer complex cells receive their input from the layer 4 simple cells. As cells in layer 4ab project to layer 2 + 3, and as, except for the bottom of layer 3, the geniculate does not project to this layer, it is likely that the receptive fields of cells in the upper layers are generated primarily by input from layer 4.

The layer 5 complex cells may form their fields from the concatenation of the fields of the superficial complex cells, as suggested by the extensive projection from the layer 2 + 3 cells into layer 5. The lateral spread of this projection can account for the increase in receptive field size from the superficial layers to layer 5. Because their apical dendrites pass through geniculate afferent zones, the layer 5 complex cells may also receive geniculate input. Evidence from serial electron microscopic reconstructions suggests, however, that they do not[50]. We do not know what are the differences in the inputs to standard and special complex cells which account for their differences in receptive field properties.

The substantial horizontal traverse of the layer 5 axon in layer 6 is an intriguing feature, as the layer 6 cells have very long receptive fields, showing summation for increased slit length up to very large values, some reaching 16° or more[11]. We have seen no other inputs to layer 6 or intrinsic connections within layer 6 that can account for this receptive field property; the input to layer 6 from the lateral geniculate nucleus is, if anything, more restricted than the geniculate input to layer 4 (refs 17, 38). Also, none of the cells within layer 6 that we have injected thus far have axons that ramify within the layer over nearly as large an area as do the axons from the layer 5 cells. This hypothesis requires us to account for elongation in both simple and complex fields in layer 6 despite the fact that the input from layer 5 is exclusively complex. The simple fields may be produced by an interaction between the geniculate input to the upper half of layer 6 and the input from layer 5.

The rich projection from layer 6 to layer 4 suggests that some properties manifested by layer 4 cells may not depend solely on convergence of geniculate input onto layer 4 cells or on connections made by those cells within the layer. It could be that, due to their intimate involvement with layer 4 through dendritic arborisation and axonal projection, the layer 6 cells have a role in producing orientation specificity, preference for direction of stimulus movement and/or end-inhibition.

1

2+3

4ab

4c

5

6
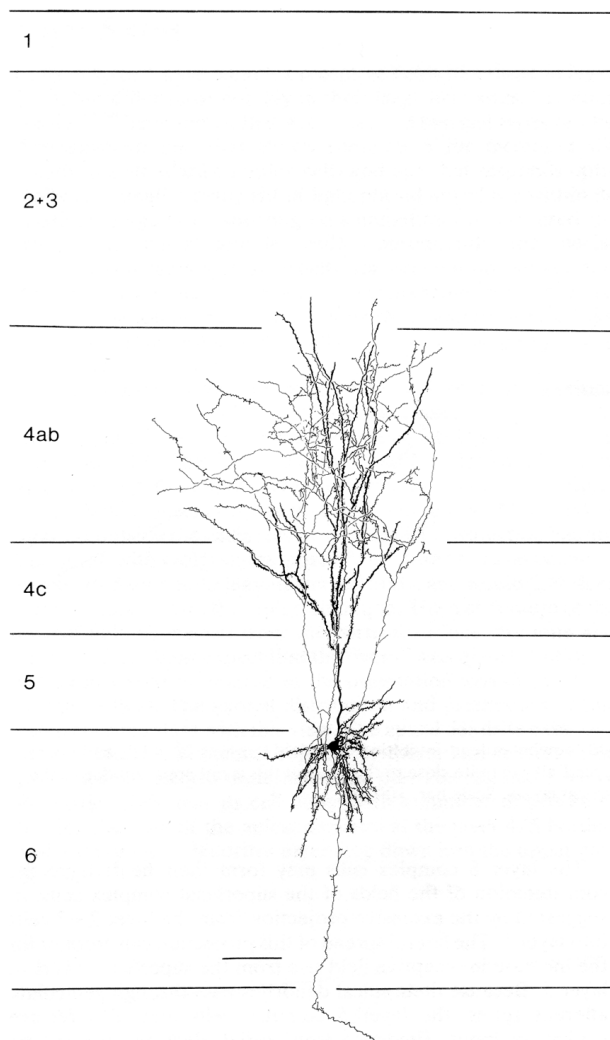
Fig. 5 A pyramidal cell in layer 6, with a simple receptive field showing the lack of end-inhibition characteristic of cells in this layer. The field size was $1\frac{1}{2}° \times 2°$, centred 4° from the area centralis. It did not show obvious summation to long slits and may represent a population of layer 6 cells with shorter fields. Scale bar, 100 μm.

Dendritic morphology and receptive field construction

Several issues are raised in considering correlations between morphology and function. As seen from our injections in layer 5, for example, large neurones have large receptive fields. This is presumably due to their large dendritic fields, which enable the cells to collect input over a wide area. Another determining factor in receptive field size is the extent of axonal ramification of the inputs to a given cell. This is seen for the layer 6 cells; although their dendritic fields are relatively small, they receive input from layer 5 over a very large area, due to the wide spread of the layer 5 cell axons. The layer 5 fields may derive their large area from both mechanisms: they have very wide dendritic arbors, and the layer 2 + 3 cells have widely arborising axons in layer 5.

A correlation between simple receptive field type and stellate morphology was found by Kelly and Van Essen[23] but, as they indicated, this was not a strict correlation. We have injected simple cells in layer 5a and 6 that were clearly pyramidal in morphology and one complex cell in layer 6 that was stellate. Thus, the simple/complex receptive field classification seems not to be the pertinent feature in relating functional properties

to the stellate/pyramidal categories. The determinant factor as to whether a pyramidal cell is simple or complex seems to be the position of its basal dendrites relative to the geniculate input. The function of apical dendrites remains unknown; they may ramify at different levels within the cortex, but so far this does not seem to correlate with any receptive field differences. It is possible that, as seen for some cells in layer 6, the role of the apical dendrite is to participate in the formation of recurrent loops within the cortex.

In addition to the dichotomy between stellate and pyramidal cells, there is, even for a given layer, considerable variability within each of these morphological classes. At this early stage in our study we do not know how this diversity is reflected in the receptive field and/or firing properties of cells. There are, on the other hand, certain receptive field features, such as orientation specificity and preference for direction of stimulus movement, for which a morphological correlate may ultimately be found. Another functional difference between cells is their inhibitory or excitatory postsynaptic effects, which depend on the transmitters they use. As mentioned above, this may be reflected in morphological differences and not in differences in receptive field properties. Pharmacological evidence suggests that inhibition is important in enhancing certain receptive field features, such as orientation specificity[51]. The relationship between function and dendritic morphology remains a challenge. We expect that further analysis of and additions to our catalogue of injected cells will reveal clues to this relationship.

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