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CELL STRUCTURE AND FUNCTION IN THE VISUAL CORTEX OF THE CAT

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SUMMARY

1. The organization of the visual cortex was studied with a technique that allows one to determine the physiology and morphology of individual cells. Micro-electrodes filled with the fluorescent dye Procion yellow were used to record intracellularly from cells in area 17 of the cat. The visual receptive field of each neurone was classified as simple, complex, or hypercomplex, and the cell was then stained by the iontophoretic injection of dye.

2. Fifty neurones were successfully examined in this way, and their structural features were compared to the varieties of cell types seen in Golgi preparations of area 17. The majority of simple units were stellate cells, whereas the majority of complex and hypercomplex units were pyramidal cells. Several neurones belonged to less common morphological types, such as double bouquet cells. Simple cells were concentrated in layer IV, hypercomplex cells in layer II+III, and complex cells in layers II+III, V and VI.

3. Electrically inexcitable cells that had high resting potentials but no impulse activity were stained and identified as glial cells. Glial cells responded to visual stimuli with slow graded depolarizations, and many of them showed a preference for a stimulus orientation similar to the optimal orientation for adjacent neurones.

4. The results show that there is a clear, but not absolute correlation between the major structural and functional classes of cells in the visual cortex. This approach, linking the physiological properties of a single cell to a given morphological type, will help in furthering our understanding of the cerebral cortex.

INTRODUCTION

The complexity of the wiring of the cerebral cortex is obvious to anyone who examines a Golgi preparation of the brain. That these intricate connexions are made with a high degree of specificity has been evident since the pioneering work of Cajal (1911). Physiological studies of single

neurones in the mammalian visual cortex have shown that these orderly connexions have a functional counterpart in the specificity of cellular responses (Hubel & Wiesel, 1962, 1965, 1968). Cortical cells typically respond best to appropriately oriented slits and edges of light within a small part of the visual field. Furthermore, they can be classified into three reasonably distinct groups, simple, complex, and hypercomplex, on the basis of the organization of their receptive fields. Many of the properties of simple cells can be accounted for by assuming a direct input from appropriate groups of fibres originating in the lateral geniculate nucleus. Complex and hypercomplex receptive fields, on the other hand, seem to be generated by further processing within the cortex.

In the primary visual cortex (area 17) the three functional classes of cells are not evenly distributed throughout the depths of the grey matter: complex units are aggregated in the superficial and deep layers of the cortex, whereas simple cells are more common in the middle layers. Hypercomplex cells, which constitute only a small percentage of the units in area 17, are concentrated in the superficial layers (Hubel & Wiesel, 1968; see below). These observations bear an interesting relationship to the cytoarchitecture of the cortex, since it is known that stellate cells are clustered in the middle layers, where afferent fibres from the lateral geniculate nucleus terminate, while pyramidal cells are more abundant in the superficial and deep zones. The suggestion therefore arises that stellate cells might have simple receptive fields and be situated close to the source of visual input. Pyramidal cells, whose axons not only ramify within the cortex but also leave it, would be good candidates for complex and hypercomplex neurones.

The present study was designed to provide direct evidence concerning this scheme. Individual cells in area 17 of the cat were first classified physiologically and then stained by the intracellular injection of the fluorescent dye Procion yellow (Stretton & Kravitz, 1968). The injected cells were then compared with the cell types found in Golgi preparations in an attempt to assign functional roles to certain structural classes of neurones.

These experiments also provided an opportunity to study the physiology and morphology of glial cells in the visual cortex by staining them while recording intracellularly. Slow depolarizations have been seen in glial cells in several preparations as a result of activity in surrounding neurones (Karahashi & Goldring, 1966; Orkand, Nicholls & Kuffler, 1966; Baylor & Nicholls, 1969). Here we have determined what kinds of visual stimuli depolarize cortical glial cells and thus have gained further insight into their functional properties. Preliminary reports of these experiments have already appeared (Van Essen & Kelly, 1973*a*, *b*).

METHODS

Adult cats were anaesthetized with ketamine hydrochloride (13 mg/kg, I.M.) supplemented during surgery with sodium thiopental (15–30 mg/kg, I.V.). Throughout the experiment the e.e.g. was monitored, and a state of light anaesthesia was maintained by a steady infusion of thiopental (2–4 mg/hr, I.P.). The cat was mounted in a stereotaxic apparatus and artificially respirated after being paralysed with succinylcholine (15 mg/kg.hr, I.V.). The concentration of CO_2 in the expired air was monitored and maintained between 3·5 and 4 %. Body temperature was kept at 38–39° C. A bilateral pneumothorax helped to reduce cortical pulsations. Since experiments often lasted more than one day, 150,000 u. penicillin G were administered I.M.

Atropine and neosynephrine were applied topically to dilate the pupils and retract the nictitating membranes, and the eyes were then focused through appropriate contact lenses on to a tangent screen placed 1·5 m in front of the animal. Visual stimuli (spots, slits, and edges of light) were shone on the screen using a modified slide projector. Receptive field maps were plotted on individual sheets of paper. Stimuli were 0·5–1·5 log units brighter than the background illumination, which was about 1cd/m^2. The projections of the optic disk and area centralis of each eye were located on the screen with the aid of a double beam ophthalmoscope. The receptive fields of all the units we studied were within 10°, and usually within 5°, of the area centralis.

A 2 × 5 mm region of area 17 (Horseley–Clarke frontal plane 0 to − 5, lateral plane 0–2) was exposed and covered with 3·5 % agar in saline. The opening in the cranium was covered with a cylindrical Lucite chamber sealed to the skull with dental wax. The sliding top plate of this closed chamber contained a small hole that formed a tight seal about the micro-electrode, which was held in an automatic stepping micromanipulator (AB Transvertex, Sweden). Appropriate positioning of the central hole allowed penetrations to be made in any region of the exposed cortex. The site of entry of each micro-electrode was marked on an enlarged photograph of the cortex using superficial blood vessels as landmarks.

In each electrode track we attempted to inject only one cell; a total of eight to twelve separate penetrations were made in most experiments, with a minimum distance of 0·5 mm between the tracks. At the end of the experiment several superficial dye marks were made by passing a 1–2 μA negative current for 10–30 sec through a low-resistance electrode filled with a 4 % solution of Chicago Blue. These marks served as reference points so that during histological reconstruction each injected cell could be reliably assigned to a particular penetration. Chicago Blue fluoresces a deep red and diffuses very little through cortical tissue; thus the dye could be seen as a localized spot with either bright field or fluorescence optics.

Finally, the animal was perfused through the heart with 10 % formol-saline, and the appropriate region of cortex was left overnight in a 10 % formalin/30 % sucrose solution. Serial coronal sections were cut at 80 μm on a freezing microtome, mounted in Lustrex, and examined with fluorescence microscopy for injected cells. When an injected cell was found, a nearby section was stained by the Nissl method to determine the layer the cell occupied and to insure that it was within area 17. We found well stained cells after injections both early on the first day and late on the second day of the experiment, indicating that the quality of cell staining is not closely dependent on the time between injection and fixation.

Electrodes. In the initial experiments we used fine-tipped Procion yellow electrodes with resistances of 300–500 MΩ. Although these electrodes gave stable penetrations of neurones they did not pass enough dye to stain the cells. Electrodes with broken

tips, having resistances of 100–200 MΩ, were more successful in staining cells but rarely gave intracellular penetrations that were stable enough to permit functional classification of a unit. Most of our injections were achieved using electrodes whose tips had been bevelled (Barrett & Graubard, 1970). The electrodes were filled by boiling in water and substituting a 6 % solution of Procion yellow M4RAN in the electrode shank. They were then bevelled by grinding against a hard Arkansas stone (L. A. Clark Co., Seattle) mounted on a small lathe. The electrodes had resistances of 20–80 MΩ and tip diameters of 1–3 μm, making it possible to obtain stable extracellular recordings from cortical cells, while the tips remained sharp enough to penetrate them. Extracellular recording was particularly helpful because often a half-hour or more was needed to complete the classification of a cell.

After an extracellularly recorded unit was classified, we attempted to impale it by advancing the electrode in 4 μm steps and by passing brief hyperpolarizing currents (10–50 nA) through the micro-electrode. If a cell was impaled, it was of course necessary to determine whether it was the same as the unit studied extracellularly. This usually took only a few seconds, since the receptive field organization and optimal stimulus requirements were already known for the externally recorded neurone. Once the identification was completed the cell was stained by passing a steady hyperpolarizing current (1–20 nA) for as long as the electrode remained intracellular (1–30 min). If currents greater than 10 nA were passed for several minutes a faint halo of extracellular dye was sometimes seen at the site of the attempted injection. The presence of extracellular marks or shattered cell fragments made it possible to assign to particular cortical layers many cells that were identified physiologically but not morphologically.

Golgi preparations. In order to compare the morphology of our sample of dye injected cells with the cell types existing in area 17 of the adult cat, we prepared a series of Golgi impregnated sections. In five cats, the visual cortex of the hemisphere contralateral to the side used for dye injections was prepared by a variant of the Golgi method employing initial fixation in aldehyde followed by chrome-osmium treatment (Lund, 1973). In addition, one cat was perfused with 1 % OsO_4 in 3·5 % $K_2Cr_2O_7$ for impregnation by the Golgi rapid technique (Morest & Morest, 1966). Coronal slices of cortex, 3 mm in thickness, were embedded in celloidin after variable periods in the impregnation solutions; 120 μm sections were then cut and mounted in Lustrex. The descriptions of cell types are based upon our own observations of Golgi material and upon those of others who have studied the visual cortex of kittens and adult cats (Cajal, 1899, 1922; O'Leary, 1941; Sholl, 1955; Poljak, 1957; Garey, 1971; LeVay, 1973).

Methods of illustration. The morphological features of injected cells and Golgi impregnated cells were recorded by preparing drawings with the aid of a Zeiss camera lucida apparatus. When the processes of an injected cell were found on more than one section, it was possible to connect the branches interrupted in sectioning by superimposing drawings of successive sections. These drawings were made using a Zeiss planachromat 100 × oil-immersion objective with a variable numerical aperture (N.A. 0·8–1·25). For the thicker Golgi sections a long working distance (0·5 mm) Zeiss Neofluar 100 × oil-immersion objective (N.A. 1·1) was helpful. Photomicrographs were taken with a Zeiss 40 × Planachromat oil-immersion objective with a variable N.A.

Cortical layering. The layering scheme we have used for area 17 of the cat is adapted from that of Otsuka & Hassler (1962):

Layer I	plexiform layer.
Layer II + III	layer of small and medium-sized pyramids.
Layer IV*ab*	layer of large stellate and medium-sized pyramidal cells.

Layer IV*c*	layer of small stellate cells.
Layer V	layer of large pyramidal cells.
Layer VI	layer of irregular pyramids.

This nomenclature emphasizes only the most prominent cell types and ignores the cellular diversity found within individual layers. In Pl. 1, a photograph of a Nissl preparation of area 17, it is possible to distinguish each of the layers, although some of the boundaries are difficult to delineate precisely. There is only a gradual transition between layers II and III, and we have treated them as a single zone. The various laminae are not fixed in thickness, but vary considerably as a result of the folding of the cortex. At the apex of the post-lateral gyrus, for example, layer VI is quite broad, while at the base of the gyrus it is reduced to a small fraction of the total thickness of the grey matter.

The present layering scheme differs in several respects from the one used by Hubel & Wiesel (1962). Their layers 2, 3 and 4 correspond respectively to layers II + III, IV*ab*, and IV*c* of Otsuka & Hassler's. The advantage of the layering plan adopted here is that it conforms to the general pattern of layers observed in other regions of the neo-cortex (Brodmann, 1909). In particular, the majority of geniculate afferents end in the subdivisions of layer IV as defined above (Rossignol & Colonnier, 1971).

RESULTS

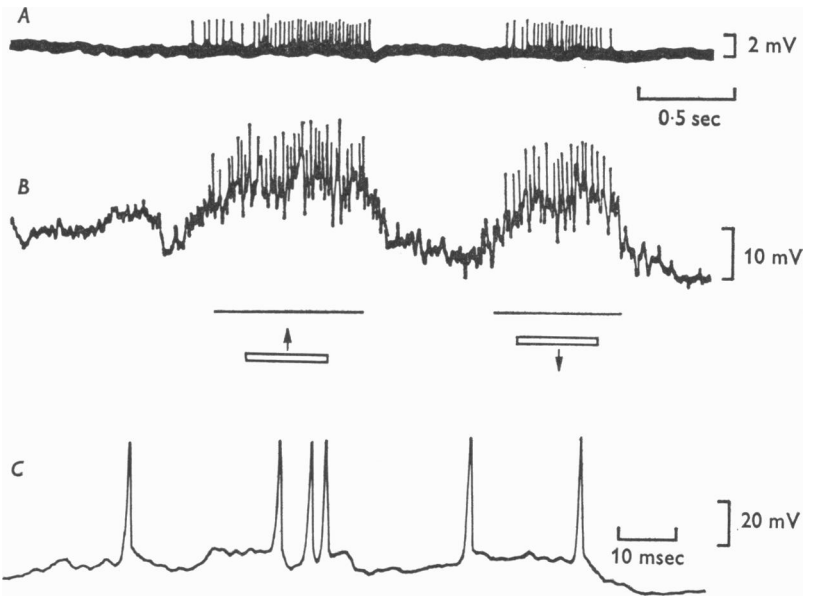
Neurones

Physiological recording and identification. In each electrode track through the grey matter it was usually necessary to record from many units in succession, either extracellularly or intracellularly, before finding a physiologically identified cell that could be impaled well enough to attempt an injection. In thirty-eight cats we recorded from more than two thousand cells, attempted to inject more than three hundred of them, and finally recovered fifty stained and identified neurones and seven glial cells.

Text-fig. 1*A* and *B* illustrates a typical sequence of extracellular and intracellular records obtained with a bevelled, dye-filled micro-electrode. This neurone had a complex receptive field and responded best to a horizontal slit moved slowly upward or downward through the receptive field. The externally recorded impulses were positive-going and about 2 mV in amplitude (Text-fig. 1*A*). When a steady hyperpolarizing current was passed through the micro-electrode, the noise level suddenly increased and the impulses reached 10–15 mV in size, indicating that the cell had been impaled. The cell still responded well to movement of the horizontal slit and the impulses could be seen to arise from a barrage of synaptic activity (Text-fig. 1*B*). Further mapping of the receptive field quickly established that the impaled neurone was identical to the extracellularly recorded unit.

The quality of the intracellular penetrations obtained with bevelled electrodes varied widely, with resting potentials and action potentials ranging in amplitude from 5 to 50 mV. We were surprised to find that

even when a penetration was obviously of poor quality, it was still possible to get a fairly good injection of the cell. Fine-tipped electrodes (300–500 MΩ resistance) were not effective in staining cells, but they did provide better intracellular penetrations, as shown in Text-fig. 1*C*. Both



Text-fig. 1. Recordings from cortical neurones with fine-tipped and bevelled micro-electrodes. *A*, an extracellular trace from a complex cell recorded with a bevelled, Procion yellow filled micro-electrode whose resistance was 30 MΩ. A burst of positive-going action potentials was elicited from the cell by a horizontal bar of light swept across the receptive field in either direction. *B*, responses of the cell after it had been impaled by passing a brief hyperpolarizing current (10 nA) through the micro-electrode. The responses to visual stimuli recorded after penetration were virtually the same as those seen extracellularly. In penetrations of this type it was usually necessary to pass a small hyperpolarizing current (0·5–5·0 nA) through the micro-electrode to prevent injury discharges. Such small currents did not grossly affect the responses to visual stimuli, but the larger currents used to stain cells (1–20 nA) greatly reduced or even abolished the evoked responses.

C, intracellular responses from a cortical cell obtained with a fine-tipped K-acetate electrode (100 MΩ resistance). The resting potential of this cell was 60 mV, and the cell did not fire spontaneously even though no current was being injected through the micro-electrode. The activity in this record was evoked by the optimal stimulus for the cell, a moving vertical slit. Large excitatory and inhibitory synaptic potentials could also be seen. The comparison between *B* and *C* illustrates the fact that fine-tipped micro-electrodes gave much better penetrations, but when filled with dye their resistances were much higher (300–500 MΩ) and they did not pass enough dye to stain cells.

spontaneous and evoked synaptic activity could be seen in this neurone, which had a resting potential of 60 mV and action potentials 55 mV in size.

Neurones were classified as simple, complex, or hypercomplex according to the criteria of Hubel & Wiesel (1962, 1965). The majority of the neurones we encountered could be classified readily along these lines, but some cells were difficult to identify functionally with complete confidence. The difficulties were usually technical, particularly when recording instability limited the time available for classifying a unit. Only a small number of cells whose classification was probable but not definite were actually stained; omission of this group from the total sample would not affect our general conclusions.

Other properties. In addition to classifying each cell as simple, complex, or hypercomplex, we often tried to obtain a fairly complete qualitative description of its properties, including the ocular dominance class, the level of spontaneous firing, the preferred directions and speeds of stimulus movement, and whether the responses to stationary stimuli were sustained or transient. Our observations, drawn from seventy cells for which detailed physiological information was available, suggest that none of these parameters provides a particularly useful index for predicting the receptive field classification of a neurone. For example, complex cells (or simple cells) can prefer either slow or fast movement, can either show a directional preference or be non-directional, and can give either sustained or transient responses (see Tables 2–4). Our finding that about two-thirds of both simple and complex cells responded best to slowly moving stimuli differs from the results of Pettigrew, Nikara & Bishop (1968), Hoffman & Stone (1971), and Stone & Dreher (1973), who state that most simple cells prefer slow movement and most complex cells prefer fast movement. This discrepancy is probably related to differences in the criteria used for receptive field classification: for example, the relative importance placed on using stationary *vs.* moving stimuli for analysing receptive fields varies from one laboratory to the next.

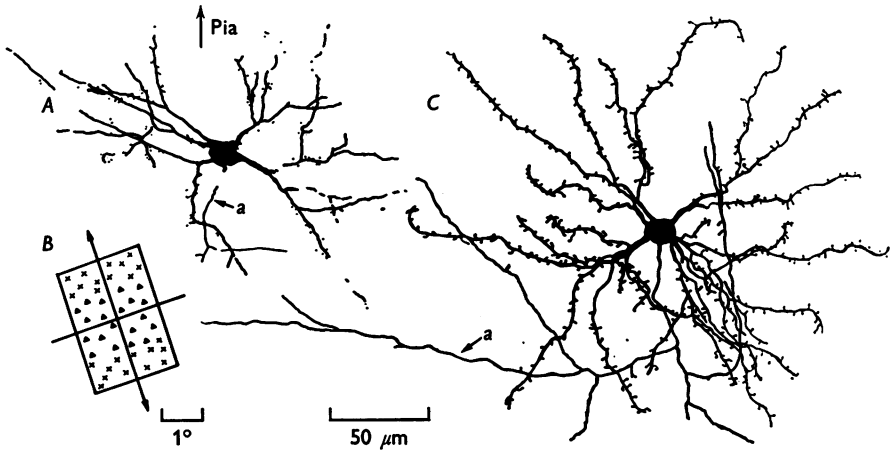
Retinal ganglion cells and lateral geniculate neurones that give sustained responses to stationary stimuli (X cells) consistently prefer slow movement, while transiently responding cells (Y cells) prefer fast movement (Cleland, Dubin & Levick, 1971). In the cortex, however, we saw only a slight tendency for these two parameters to be linked: eleven of fifteen sustained units responded best to slow movement ($< 2°$/sec), while seventeen of thirty-one transient cells preferred moderate (2–10°/sec) or fast ($> 10°$/sec) movement. The only consistent patterns we found were that hypercomplex cells always gave transient responses and that all cells with high spontaneous activity (about 10 % of the total sample) were complex cells, usually in layer V.

Examples of dye injected neurones. The major findings of this study (see Table 1) can best be appreciated by considering the properties of a few well stained cells. This will illustrate the outcome of the experiments in a qualitative way and bring some of the issues involved into sharper relief.

The first cell to be described was a simple cell stimulated maximally by a narrow slit of light swept across the field at about 30° from the horizontal (i.e. a 2:00–8:00 orientation). When the receptive field was mapped with small stationary spots of light, it could be divided into a central 'off' zone flanked by two antagonistic 'on' zones (Text-fig. 2*B*). A stronger response

was produced by increasing the size of the stimulus, provided it was confined to one zone and did not impinge upon an antagonistic area.

After injection with Procion yellow, this simple cell was found to have a rounded cell body about 12–15 μm in diameter (Pl. 2). Comparison with an adjacent Nissl-stained section showed that the cell was in layer IV*ab*. Several processes radiate in various directions from the soma and run out of the plane of focus. A more complete representation of the morphology of this cell is provided by the camera lucida drawing of Text-fig. 2*A*. The



Text-fig. 2. An injected simple cell in layer IV*ab* and a Golgi impregnated spiny stellate cell from the same layer are shown here for comparison. *A*, camera lucida drawing of the injected simple cell in Pl. 2. It is a spiny stellate cell whose cell body lies in layer IV*ab*. Some of the processes in the drawing are interrupted either because the branch was too faintly stained to be traced along its entire length or because the drawings for adjacent sections were not in perfect register when superimposed. Most processes bear dendritic spines, but one is smoother in contour and is probably the axon (*a*). *B*, receptive field map of this cell; an × indicates an 'on' response to a small spot of light, a △ indicates an 'off' response. The receptive field was divided into antagonistic 'on' and 'off' zones, a characteristic property of simple cells. *C*, a drawing of a Golgi impregnated spiny stellate cell from the same layer. The size of the cell body, the distribution of spiny dendrites and the initial downward course of the axon are similar in the two cells.

dendrites, which bear spines, divide repeatedly in the vicinity of the cell body. One process, whose connexion with the cell body could not be detected, is smoother in contour and is probably a descending axon that emits collateral branches. This neurone is clearly a spiny stellate cell, as can be seen by comparing it to a drawing of such a cell taken from a Golgi preparation (Text-fig. 2*C*). Stellate cells are characterized by a more or less spherical cell body and by dendrites that usually show no preferred

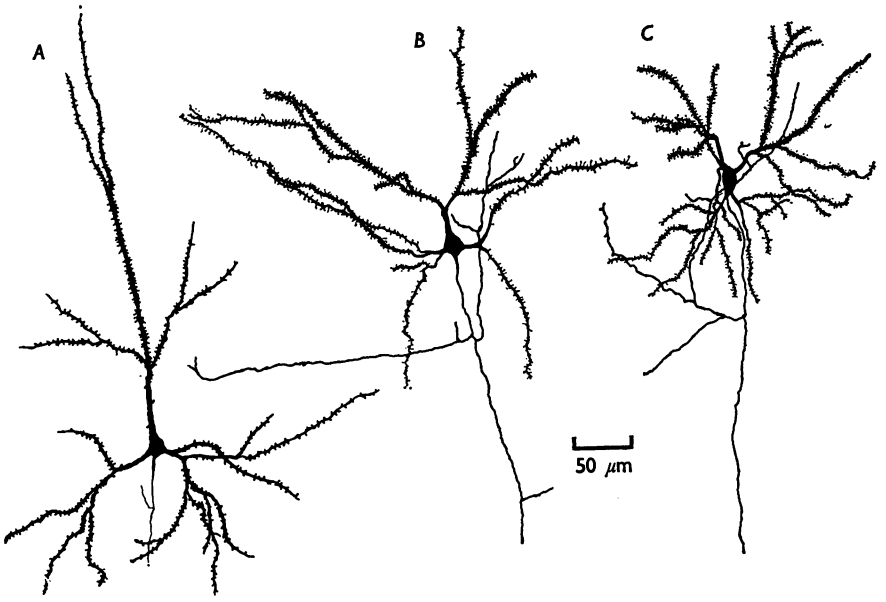
orientation and are restricted in distribution, often to a single layer. The axon usually terminates locally in an extensive ramification. The two major types of stellate neurones, spiny and spine-free, are distributed differently within the cortex. Spiny stellate cells are confined principally to layer IV, whereas spine-free or sparsely spined cells are found in all cortical laminae (Lund, 1973; LeVay, 1973).

A photomicrograph of an injected complex cell is shown in Pl. 3. This unit responded best to moving slits and edges of light held in a 4:00–10:00 orientation and gave brisk discharges when stationary slits were flashed on or off anywhere within the receptive field. There was little or no response to small spots, improperly oriented slits, or uniform illumination of the field, indicating that the cell had typical complex receptive field properties. Injection of this cell revealed a conical cell body, 8–10 μm in diameter, lying in layer V. The soma gradually tapers to an apical dendrite that could be followed on an adjacent section to a point of bifurcation in layer IV. A plexus of basal dendrites emanates from the cell body and ramifies within layer V. This cell is plainly pyramidal, even though it was not intensely enough stained to permit dendritic spines to be seen or the axon to be identified. This is borne out by comparing the injected cell to a camera lucida drawing of a Golgi impregnated pyramidal cell from the same layer (Text-fig. 3*A*). Pyramidal cells have an ascending dendrite that usually branches within layer I, numerous basal dendrites and a long axon that descends to the white matter. The dendrites of these cells are studded with spines over much of their extent. Pyramidal cells are found in layers II through VI of the cortex, but are least common in IVc; variations in the shape of pyramidal cells from layer to layer are illustrated in Text-fig. 3.

The next injected cell is a hypercomplex unit that responded well to a short vertical slit, about $\frac{1}{8}° \times \frac{1}{2}°$, but not to a long vertical slit or to a horizontal slit whatever its length. The excitatory portion of the receptive field was quite small, only $\frac{1}{2}° \times \frac{3}{4}°$. A photomicrograph of this cell (Pl. 4*A*) shows a triangular cell body in layer II + III and an apical dendrite that bifurcates just above the cell body and sends both branches toward layer I. The initial segment of the axon can be seen descending from the base of the soma. Although it differs in some respects from the layer V pyramid shown in Pl. 3, this cell resembles many of the pyramidal cells seen in layer II + III (e.g. Text-fig. 3*B*).

The identification of each of these injected cells was clear-cut on both morphological and physiological grounds. But not all injected cells were easy to identify morphologically, even when their processes were relatively well stained. For example, Text-fig. 4*B* is a camera lucida drawing of an injected complex cell from layer V. Numerous dendritic spines can be seen

on the processes that arise from all aspects of the large (20 μm in diameter) cell body, but no apical dendrite stands out distinctly. This cell is not a typical pyramid, nor is it one of the layer V stellate cells, which are generally smaller and spine-free. It is most likely a type of irregular pyramid called a multiform pyramid (Text-fig. 4*C*). These cells, found in the deeper layers of the cortex, have a polygonal cell body and many spiny dendrites. One of the dendrites, which may be small in diameter and often arises from the flank of the cell body, turns upward to reach layer I. The

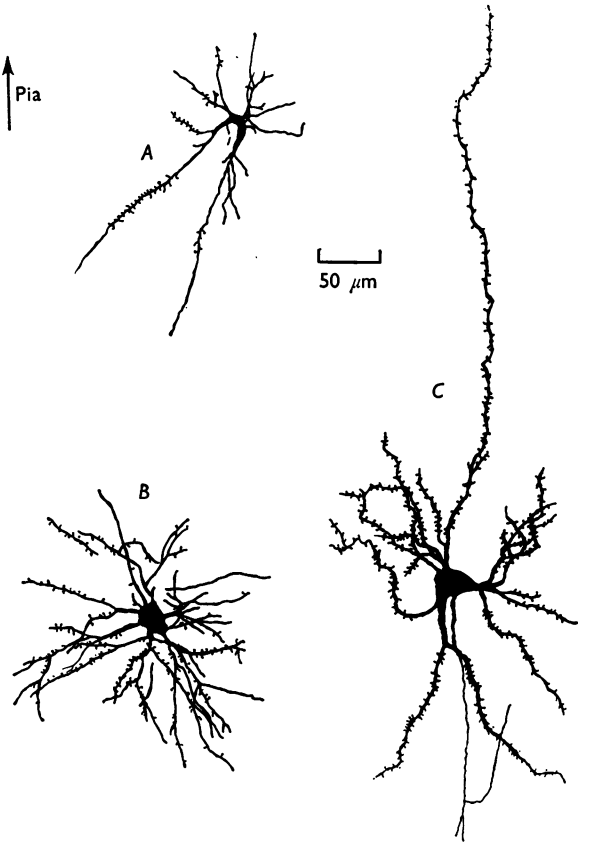


Text-fig. 3. Golgi impregnated pyramidal cells from the deep and superficial layers of the cortex. Cell *A* is a small layer V pyramid, easily identified because of the shape of the cell body, the course of the axon and the distribution of dendrites. Cell *B*, from layer II, is a pyramid whose apical dendrite bifurcates near the cell body to send branches into layer I. Cell *C* is not overtly pyramidal in shape at all but it possesses the three principal features of the pyramidal class; *a*, spiny dendrites, *b*, branches in layer I, and *c*, a long descending axon. Note that if the cell depicted in *C* were not well injected, it could easily be mistaken for a stellate cell.

axon descends to enter the white matter. In short, multiform cells share the important properties of pyramids: spines, a dendrite that reaches layer I, and a long descending axon.

A small percentage of cortical neurones clearly do not fit into the categories of either stellate or pyramidal cells. There are several varieties of such exceptional cells, one of which is shown in Text-fig. 4*A*. This is a drawing of an injected simple cell whose cell body lies in layer V. Two

spiny dendrites originate from either pole of the crescent-shaped cell soma and descend obliquely towards the white matter. Several smaller dendrites arise from other parts of the cell, but the axon could not be identified. The few such 'crescent' cells we have seen in Golgi preparations have also been in layer V and have had ascending axons.



Text-fig. 4. *A* and *B* are injected cells that were not easy to identify morphologically. *A*, an injected 'crescent cell' from layer V that had simple receptive field properties. Two spiny dendrites emerge from the cell body and run obliquely toward the white matter. A number of other dendrites can be seen, but the axon was not clearly visible.

B, an injected complex cell from layer V. Numerous spiny dendrites radiate in all directions from the large (20 μm) cell soma. *C*, a Golgi impregnated multiform pyramid is shown here for comparison with *B*. Note that the size of the irregularly shaped cell body and the distribution of the dendrites are roughly similar in the two cells, although the injected cell was not stained well enough to detect a clear apical process. Because of the similarities between these two cells, and because spiny stellate cells are not found in layer V, cell *B* was classified as an irregular pyramid.

In layer II + III it is not always possible to distinguish between stellate and pyramidal cells on the basis of dendritic geometry alone. For example, the cell in Text-fig. 3*C* has an irregular shape but can be identified as a pyramid because it has spines, dendrites in layer I, and a long axon. Several of the injected neurones had a similar shape (see Text-fig. 11*C*, *D*), but because spines and axonal processes were not visible these cells could not be classified with confidence. To deal with problems such as this we have adopted a category of 'other' injected types, which includes the crescent cell in Text-fig. 4*A*, the double bouquet cell described below, and the cells that could not be easily identified as either stellate or pyramidal.

TABLE 1. Neurones injected in area 17

	Simple	Complex	Hypercomplex
Stellate	8	1	1
Pyramidal*	3	23	5
Other†	2	4	1

* Includes irregular pyramids.

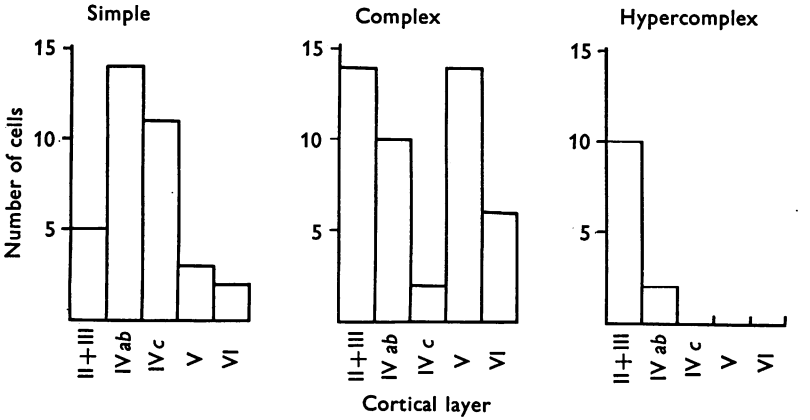
† Includes double bouquet cells, crescent cells, and neurones not readily classifiable as stellate or pyramidal.

Overall results for injected neurones. More than half (twenty-eight) of the fifty successfully stained and identified neurones had complex receptive fields; thirteen others were simple and seven were hypercomplex. Of the remaining two units one had intermediate physiological properties and the other was non-oriented (see below). Table 1 summarizes the relationship between the major functional and structural classes of cells. Most simple cells were stellate (eight of thirteen), and most complex (twenty-three of twenty-eight) and hypercomplex (five of seven) units were either pyramids or irregular pyramids. The general pattern is even more apparent when viewed from a different perspective: eight of the ten stellate cells, but only three of the thirty-two pyramids, had simple receptive fields.

Laminar organization. The three functional classes of neurones are not distributed evenly within the various cortical layers. Text-fig. 5 shows the laminar distribution of ninety-three physiologically identified cells, including the forty-eight successfully stained simple, complex, and hypercomplex units and an additional forty-five cells whose positions were marked by cell fragments or extracellular dye marks. These results confirm the earlier observations of Hubel & Wiesel (1962) that simple cells occur mainly in the middle layers of the cortex while complex cells are concentrated above and below. In addition, they show that hypercomplex cells, which form only a small minority of the cells in area 17, are restricted to the superficial cortical layers.

Because of the importance of cortical layering in the organization of area 17 we shall present the detailed results layer by layer, starting with layer IV, the principal site of termination of geniculate afferents (Wilson & Cragg, 1967; Rossignol & Colonnier, 1971).

Layer IV. In layer IV as a whole seven simple and three complex cells were stained. Six of the seven simple cells were stellate, three in layer IV*ab* and three in IV*c* (Text-figs. 2*A* and 6*A*, *B* and *D–F*); the seventh

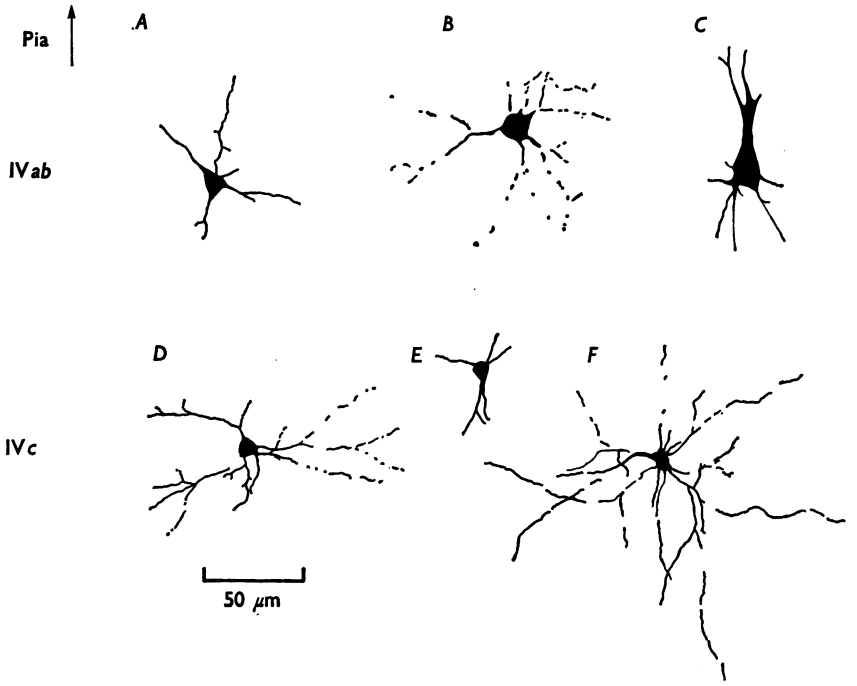


Text-fig. 5. Laminar distribution of thirty-five simple, forty-six complex, and twelve hypercomplex cells. Each histogram includes both the cells that were successfully stained and the cells whose positions were marked only by cell fragments or extracellular dye. No neurones in layer I were successfully marked. The three neurones not included in these histograms are a non-oriented unit in layer V, and two cells with properties intermediate between simple and complex cells, one in layer IV*c* and one in layer VI.

was clearly a pyramidal cell in IV*ab* (Text-fig. 6*C*). One of these simple units was a spiny stellate cell (Text-fig. 2*A*), but the other five stellate cells were too faintly stained to determine the subclass to which they belonged.

Three complex cells in layer IV*ab* were stained. One of them (Text-fig. 7*A*) was a pyramidal cell with a descending axon and numerous dendritic spines. The second complex cell in this layer (Text-fig. 7*B*) was not well injected, but more than a dozen fine processes could be seen extending in all directions from the triangular soma; it is probably either an irregular pyramid or a large stellate cell with an unusual number of dendrites. The third complex cell (Text-fig. 7*C*) had a small cell body and numerous varicose processes extending mostly in a radial orientation. Cells like this were first seen in Golgi preparations by Cajal (1911), who termed them double bouquet cells. These cells lack spines and their axons do not enter

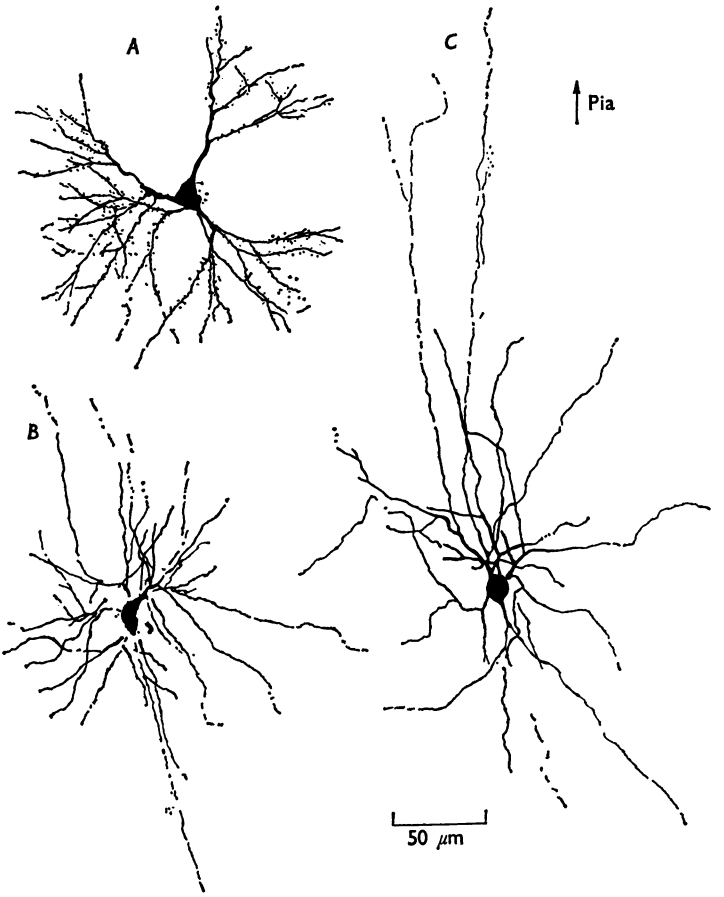
the white matter; in these respects they are similar to spine-free stellate cells. The most extreme examples of double bouquet cells have dendritic and axonal branches running strictly in parallel, but other neurones appear to be intermediate between well defined stellate and double bouquet cells.



Text-fig. 6. Camera lucida drawings of injected simple cells in layer IV. *A* and *B*, stellate cells in layer IV*ab*. *C*, a pyramidal cell in IV*ab* whose physiological properties were not obviously different from the injected simple stellate cells. *D*, *E* and *F*, stellate cells in layer IV*c*. All of these cells were rather faintly stained, and neither spines nor axonal processes could be seen in any of them. The receptive field classification of cell *D* was probable, but not definite. Table 2 presents further physiological data about these cells.

The physiological properties of the ten injected neurones in layer IV are shown in Table 2. No consistent pattern was seen relating the various physiological parameters either to the original classification of cells as simple or complex or to the morphology determined after injection. The one simple pyramid was not obviously different in its physiology from the simple stellate cells. There was a tendency in this small sample for sustained cells to occur in layer IV*c* and transient cells in IV*ab*, but this trend was not apparent after including the cells that were localized to one of these layers by extracellular dye marks.

Layers V and VI. The results for the twenty-one neurones stained in the deeper layers were clear cut in several respects. All of the sixteen complex cells were classified as pyramidal. Eleven were typical pyramids (Pl. 3 and Text-fig. 8), and the other five were irregular pyramids. Two of the irregular pyramids were triangular in shape (Text-fig. 9*A*, *C*) and the other three were multiform cells (Text-figs. 4*B* and 9*B*, *D*).



Text-fig. 7. Injected complex cells in layer IV*ab*. *A*, a pyramidal cell with numerous dendritic spines and a descending axon. *B*, a faintly stained cell that is probably either an irregular pyramid or a large stellate cell. *C*, a probable double bouquet cell.

Only three simple cells were stained in layers V and VI, and none of them were pyramidal. One was a stellate cell (Text-fig. 10*A*), another was the crescent cell described earlier (Text-fig. 4*A*), and the third was a

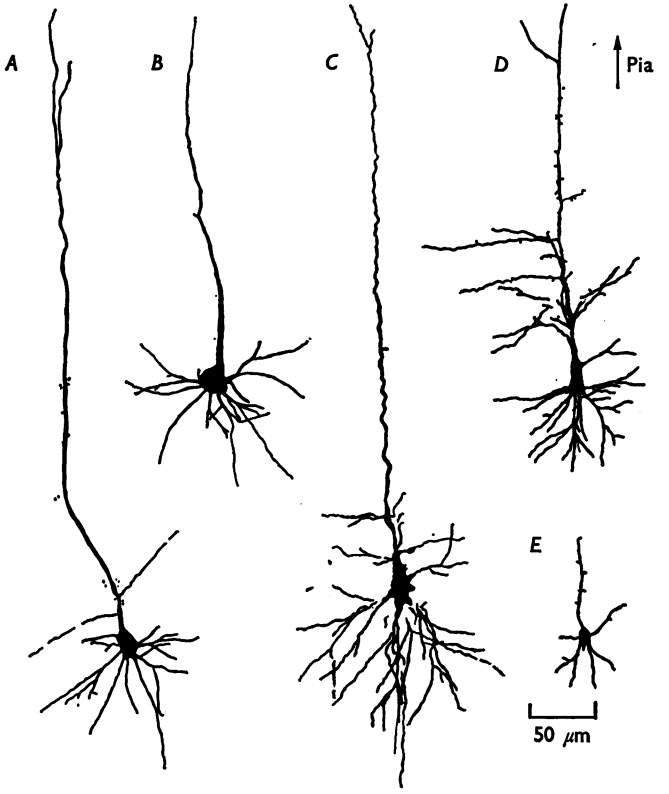
TABLE 2. Properties of injected cells in layer IV

Cell (Fig.)	Class		r.f. area (deg²)	Zones	O.D.	Direction-ality	Optimal speed	Sustained/ transient	Spontaneous firing
	Phys.	Morph.							
IV*ab*									
2*A*	S	St	7	3	—	0	—	—	+
6*A*	S	St	2	3	4	+ +	F	Tr	+
6*B*	S	St	1	2	4	+ +	S	Tr	+
6*C*	S	Pyr	3	3	—	+	F	Tr	+
IV*c*									
6*D*	S	St	5	—	—	—	—	—	—
6*E*	S	St	2	2	—	+ +	—	Sus	+
6*F*	S	St	2	3	1	0	S	Sus	+
IV*ab*									
7*A*	Cx	Pyr	5	1	5	+	—	—	—
7*C*	Cx	DB	6	1	4	+	S	Tr	+
7*B*	Cx	other	6	1	7	0	S	Sus	+

In this and the following tables, physiological class is indicated by S for simple, Cx for complex, Hcx for hypercomplex, morphological class by St for stellate, Pyr for pyramidal, and DB for double bouquet cell. The left-hand column refers to the Text-fig. in which the cell is illustrated. Receptive field (r.f.) area is usually shown to the nearest degree, and zones refer to the number of separate on, off, or on/off regions within the field. Ocular dominance (O.D.) groupings follow the conventions of Hubel & Wiesel (1962): groups 1 and 7 indicate complete contralateral and ipsilateral dominance respectively; group 4 cells are driven equally by the two eyes. Directionality: 0, none; +, slight; + +, moderate; and + + +, strong directional preference. Optimal stimulus speed: S, slow (< 2°/sec); M, moderate (2–10°/sec); F, fast (> 10°/sec). Cells were classified as sustained (Sus) if the response to an optimal stationary stimulus lasted longer than one sec, and transient (Tr) if the responses were shorter. Spontaneous firing: 0, none; +, low rate (< 1/sec); + +, moderate rate (1–10/sec); + + +, high rate (> 10/sec). A dash (—) indicates that this piece of information was not recorded for the cell in question.

faintly stained cell that had several fine processes streaming apically and might have been a double bouquet cell (Text-fig. 10*B*).

One of the cells injected in layer VI was a pyramid whose functional properties were intermediate between those of typical simple and complex cells (Text-fig. 10*D*). Its receptive field consisted of a narrow 'on' strip flanked by two antagonistic zones, an arrangement typical of simple fields.

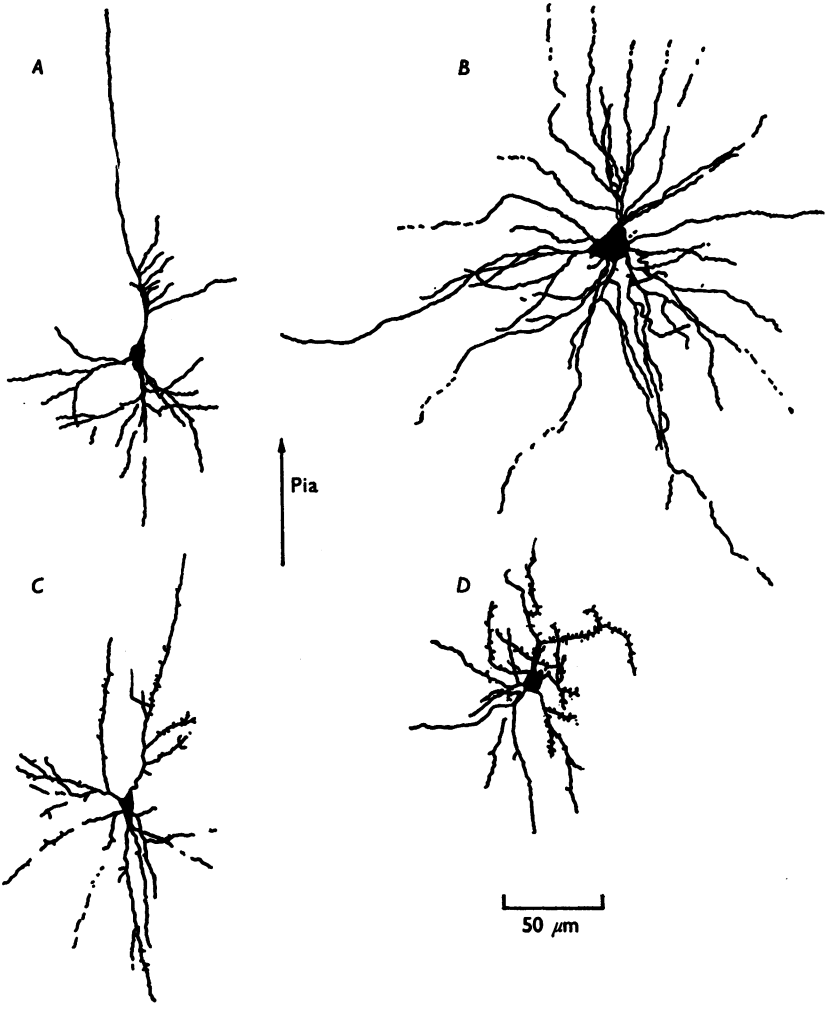


Text-fig. 8. Complex pyramidal cells injected in layers V and VI. *A–D*, large pyramids in layer V. The apical dendrites of *A* and *C* could be traced up to layer II + III before they became too faint to follow. *E*, a small pyramidal cell in layer VI. In addition to these cells and the cell in Pl. 3, six other complex pyramids were stained in these two layers, three in layer V and three in layer VI.

Narrow slits flashed on to either of the flanks gave 'off' responses, but wider slits or edges, still confined to the flanks, gave little or no response; in this respect the cell showed complex properties. One other cell with similar intermediate properties was localized to layer IV*c*.

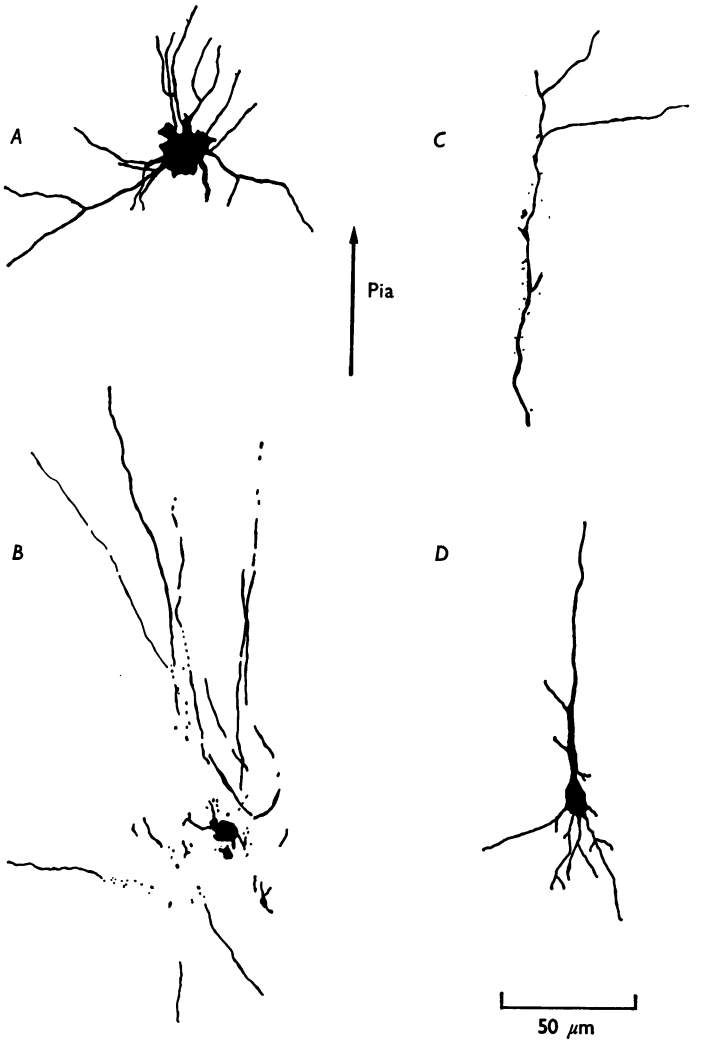
Finally, one of the units in layer V had a receptive field with a concentric centre-surround organization resembling that of a lateral genicu-

late neurone (Hubel & Wiesel 1961). The only part of the cell that was stained was a fine process, 1–2 μm in diameter, having the appearance of an axon terminal or pre-terminal (Text-fig. 10*C*). At the time of the injection we noted that the frequency of the responses to visual stimuli was unaffected by passing current through the electrode, even though



Text-fig. 9. Irregular pyramidal cells injected in layers V and VI. All of these cells had complex receptive field properties. *A*, a triangular type layer V pyramid with dendrites directed toward both the pia and the white matter. *B*, a large multiform cell in layer V. *C*, another triangular type pyramid in layer VI with distinct spines and a descending axon. Its receptive field classification was probable, but not definite. *D*, a small, spiny multiform cell in layer VI.

current did block the injury discharges of the cell. This indicated that the evoked responses originated at a considerable distance from the site of the penetration and supported the idea that the fibre was of geniculate origin. Similar observations were made for several other units with centre-surround receptive fields typical of geniculate afferents, but never for



Text-fig. 10. Other injected neurones in layers V and VI. *A*, a simple stellate cell. Cell *B*, a probable simple cell, was shattered, but it may be a double bouquet cell. *C*, an injected axonal process in layer V; its receptive field was arranged in a concentric centre-surround fashion typical of geniculate afferents. *D*, a small pyramidal cell that had properties intermediate between those of typical simple and complex cells.

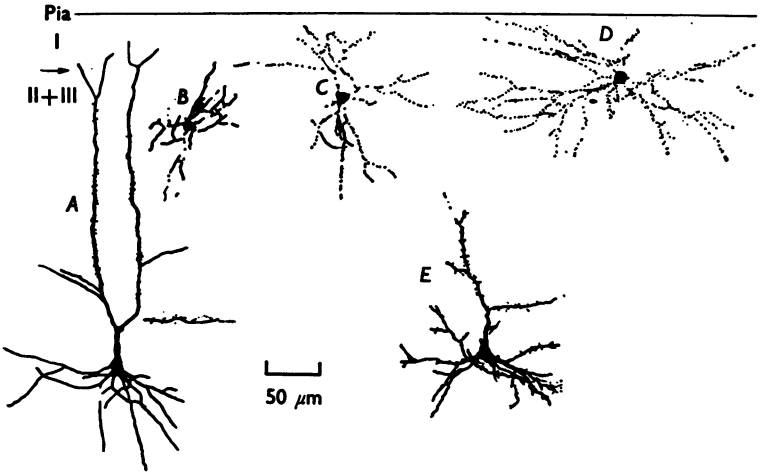
TABLE 3. Properties of injected cells in layers V and VI

Cell (Fig.)	Class: Phys.	Class: Morph.	r.f. area (deg²)	Zones	O.D.	Direction-ality	Optimal speed	Sustained/ transient	Spontaneous firing
V									
10*A*	S	St	4·5	3	3	0	F	—	+
10*B*	S	other	9	2	4	+ + +	—	—	—
4*A*	S	Cres	5	3	3	0	M	Tr	+
8*A*	Cx	Pyr	4	1	3	+ + +	M	Tr	+ +
8*B*	Cx	Pyr	4	1	2	+	S	Tr	+ +
8*C*	Cx	Pyr	3·5	1	4	+ +	S	—	—
8*D*	Cx	Pyr	15	1	—	0	S	—	+ + +
9*A*	Cx	Irr pyr	4	2	7	0	S	Tr	+
9*B*	Cx	Irr pyr	12	1	4	+ + +	S	Sus	0
4*B*	Cx	Irr pyr	20	1	—	—	—	—	—
Pl. 3	Cx	Pyr	12	1	4	0	—	—	+ + +
10*C*	Conc	Axon	2	2	1	0	—	—	+ +
VI									
8*E*	Cx	Pyr	9	1	4	+ +	S	Tr	+ +
9*C*	Cx	Irr pyr	7	1	—	—	—	—	—
9*D*	Cx	Irr pyr	10	1	—	0	—	—	—
10*D*	Int	Pyr	4	3	—	+	—	—	—

Symbols explained in Table 2. In addition, Conc stands for concentric, centre-surround receptive field, Int for intermediate, Cres for crescent and Irr pyr for irregular pyramid.

units that on both physiological and morphological grounds were clearly cortical neurones.

Table 3 shows the functional properties of the cells stained in layers V and VI. Three of the complex cells injected in layer V were similar in their properties to layer V cells that project to the superior colliculus (Palmer, Rosenquist & Sprague, 1972): they were binocularly driven, showed directional selectivity, and responded as well or nearly as well to small spots as to properly oriented long slits. All three of these cortico-tectal-like cells were large pyramids; one was irregular (Text-fig. 9*B*) and two were more typical pyramids (Text-fig. 8*A*). In contrast to cells in other cortical layers, about half of the complex units in layer V had a high rate of spontaneous firing.

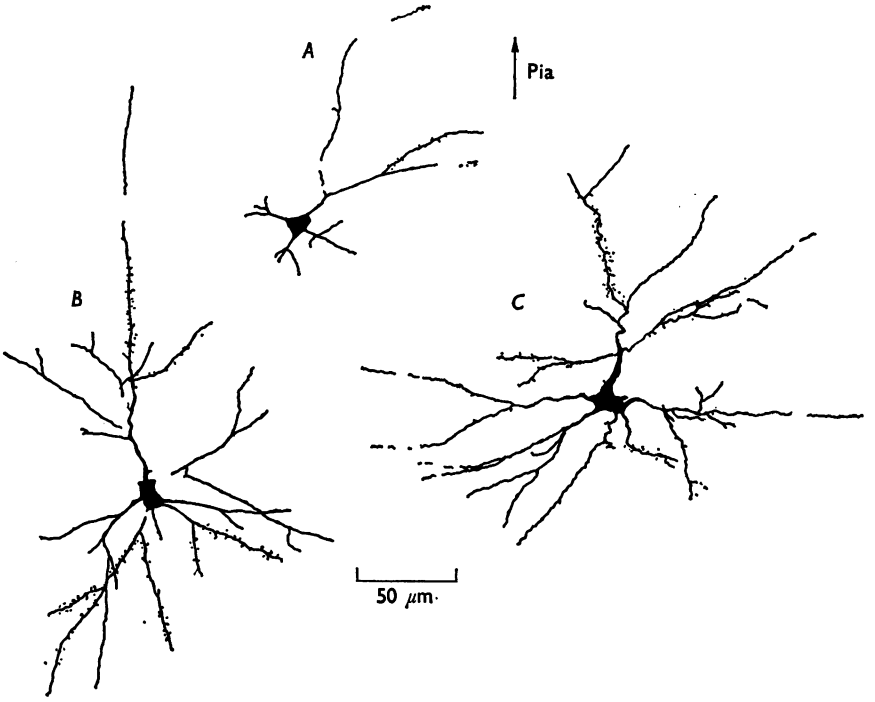


Text-fig. 11. Injected complex cells in layer II + III. The positions of these cells in the figure accurately reflect their depth from the pial surface. *A* and *E*, pyramidal cells in the deeper portion of layer II + III. *B*, a small stellate cell whose dendrites could not be traced into layer I. *C* and *D*, superficial cells whose cell bodies are just below layer I. Inasmuch as their dendrites branch extensively within layer I, it is quite possible that these cells are irregular pyramids. However, they were not classified as stellate or pyramidal because they were not well enough stained to see spines or axons. The receptive field classification of cell *E* was probable but not definite. Three other complex pyramids in layer II + III were successfully stained besides the ones shown.

Layer II + III. Six of the nine complex cells in this layer were pyramidal (e.g. Text-fig. 11*A*, *E*) and one was classified as stellate (Text-fig. 11*B*). The other two cells (Text-fig. 11*C*, *D*) were not stained well enough to see spines or axons and could not be classified with certainty because in this layer it is possible to confuse stellate cells with pyramids that have short

apical dendrites and irregularly shaped cell bodies. For this reason these two cells were placed in the category of 'other' types (see Table 1).

One of the three simple units was a small cell with a typical stellate appearance and without any visible dendrites in layer I (Text-fig. 12*A*). The other two were pyramids situated close to the border between layers II + III and IV*ab* (Text-fig. 12*B*, *C*). The morphological differences found between simple and complex cells in the deeper layers are thus not apparent in this small sample of superficial cells.

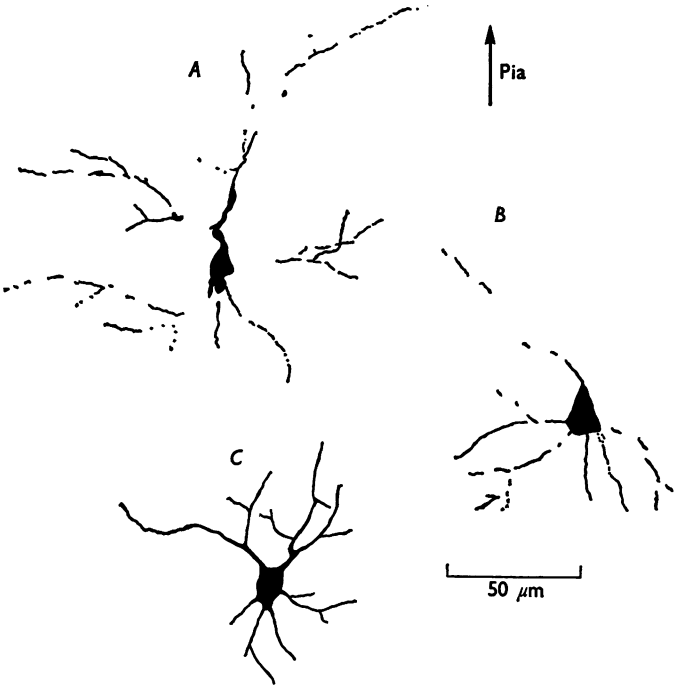


Text-fig. 12. Injected simple cells in layer II + III. *A*, a stellate cell with a few spines visible on one dendrite, which was brightly stained and thus was probably the site of the electrode penetration. *B*, a pyramidal cell situated close to the border with layer IV*ab*. *C*, a well stained cell close to the border with layer IV*ab*. In spite of the irregularly shaped cell body this cell was classified as pyramidal because of its apical dendrite and the presence of dendritic spines. Its receptive field classification was probable, but not definite.

Five of the seven hypercomplex cells in layer II + III were classified as pyramidal (Text-fig. 13*A*, *B* and Pl. 4*A*), although two were faintly stained and could not be identified with certainty. One of the hypercomplex cells was stellate (Text-fig. 13*C*) and the remaining cell was left

in the category of 'other' types because of the difficulty in distinguishing between superficial pyramids and stellate cells.

The superficial cells we encountered all had low spontaneous activity and gave only transient responses to stationary stimuli (Table 4). Some cells, particularly hypercomplex units, responded poorly or not at all to stationary stimuli. We were able to determine the spatial organization of the receptive field for only six hypercomplex neurones. Three of these cells gave only on-off responses to properly oriented short slits, suggesting that



Text-fig. 13. Injected hypercomplex cells in layer II + III. *A* and *B*, probable pyramidal cells close to layer I. *C*, a stellate cell close to the border with layer IV*ab*. In addition to these cells and the cell in Pl. 4*A*, three other hypercomplex cells were injected; two were pyramidal and one belonged to the class of 'other' cell types.

the activating region of the receptive field was organized in a typical complex manner. The other three cells had activating regions with separate on and off zones similar to those of typical simple cells. Hypercomplex receptive fields were on the average smaller in size than either simple or complex fields.

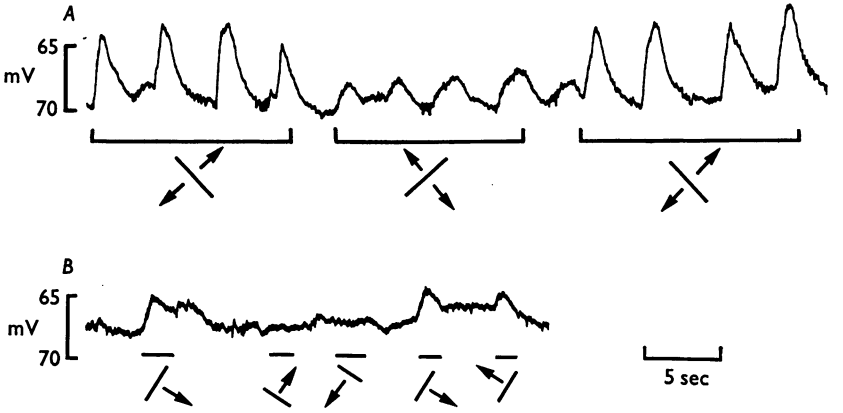
TABLE 4. Properties of injected cells in layer II + III

Cell (Fig.)	Class		r.f. area (deg^2)	Zones	O.D.	Direction-ality	Optimal speed	Sustained/ transient	Spontaneous firing
	Phys.	Morph.							
12*A*	S	St	2	3	7	+ + +	S	Tr	0
12*B*	S	Pyr	2	2	5	+ + +	M	—	—
12*C*	S	Pyr	5	2	7	+ + +	M	—	—
11*A*	Cx	Pyr	9	2	4	+ + +	F	Tr	0
11*B*	Cx	St	18	1	3	0	M	Tr	+
11*C*	Cx	other	7	1	1	0	M	—	—
11*D*	Cx	other	3	1	3	0	M	Tr	—
11*E*	Cx	Pyr	—	1	—	—	—	—	—
13*A*	Hcx	Pyr	6	1	2	0	M	Tr	—
13*B*	Hcx	Pyr	3	1	1	+ + +	F	—	—
13*C*	Hcx	St	0·5	1	7	+ + +	S	Tr	0
Pl. 4*A*	Hcx	Pyr	0·4	1	2	+ + +	M	Tr	+

Symbols are explained in Table 2.

Glial cells

Morphology. During these experiments we frequently penetrated cells that had high resting potentials (60–90 mV) but showed no signs of synaptic activity or impulse activity, even in response to depolarizing currents. These properties are common to glial cells in a variety of preparations (Kuffler & Potter, 1964; Karahashi & Goldring, 1966; Kuffler, Nicholls & Orkand 1966; Dennis & Gerschenfeld, 1969). Despite the high resting potentials, penetrations of inexcitable cells usually lasted for only a minute or two, and we were able to stain successfully only seven of these cells. All of them had the appearance of faintly stained glial cells and were unlike any of the injected neurones. One inexcitable cell (Pl. 4*B*) was spindle shaped and aligned parallel to the radial fibre bundles that run through layer V. A single process can be seen emerging from either pole of



Text-fig. 14. Visually evoked responses in glial cells. *A*, slow depolarizing responses of a glial cell to moving slits of different orientations. Left and right portions: 5–7 mV depolarizations produced by slits in a 4:00–10:00 orientation and moved alternately back and forth across the receptive field. This cell had a 70 mV resting potential and showed no signs of impulse activity. Centre: responses to the orthogonal stimulus (slits held at 1:00–7:00) are reduced to 2–3 mV. Neurones encountered in the same electrode track had the same orientation preference as the glial cell. This cell was stained and found to be an astrocyte (Pl. 4*C*). *B*, intracellular records from another glial cell that gave a 3 mV depolarization to 1:00–7:00 slits but no measurable response to 4:00–10:00 slits.

the cell soma, which is 6 μm in diameter. The size of the cell, its radial orientation, and the pattern of branching suggest that it is an oligodendrocyte. Pl. 4*C* shows another example; this cell has a small body (5 μm in diameter) surrounded by a halo of fine particles and filaments

and is probably a protoplasmic astrocyte. Five other cells were classified as astrocytes but were not stained well enough to permit a distinction between fibrous and protoplasmic subtypes.

Physiological responses. Most glial cells from which we obtained stable recordings responded to appropriate visual stimuli with slow graded depolarizations, 1–7 mV in amplitude. These glial depolarizations are presumably caused by K release during activity of surrounding neurones (see Discussion), and as such they may provide insights about the way cortical neurones are grouped together. The nature of the responses can be illustrated by an experiment in which the activities of one glial cell and two nearby neurones were recorded along a single electrode track. The first and third units in this sequence, separated by approximately 200 μm, were extracellularly recorded neurones that preferred stimuli with a 4:00–10:00 orientation. The second unit was a glial cell with a stable resting potential of 70 mV, whose receptive field position and preferred orientation were approximately the same as those of the two adjacent neurones. A slit of light in a 4:00–10:00 orientation evoked a slow depolarization, 5–7 mV in amplitude, when swept alternately up and down in a small part of the visual field (Text-fig. 14*A*, left and right). The responses to the orthogonal stimulus (1:00–7:00) were much smaller (Text-fig. 14*A*, centre) but were quite distinct even though this stimulus was ineffective in driving either of the two adjacent neurones. A few glial cells showed still more marked orientation selectivity; a record from a cell that gave a 3 mV response to one stimulus orientation, but none to the orthogonal orientation, is shown in Text-fig. 14*B*.

Visually evoked depolarizations were seen in thirty-nine glial cells, including three of the injected astrocytes. Fourteen cells, including the other three astrocytes and the one injected oligodendrocyte, gave no detectable responses to any of a variety of visual stimuli. Twelve of the responsive cells showed an orientation preference, and for six of this group one or more nearby neurones with the same optimal orientation was encountered. Five cells appeared to be non-oriented. The orientation selectivity of the remaining twenty-two responsive cells was not determined because of recording instability.

The region of the visual field over which stimuli could elicit glial depolarizations varied from one cell to the next, but in general it was somewhat larger than the individual receptive fields of surrounding neurones. Glial cell receptive fields ranged from 6 to 25 deg.^{2} in area. Some cells had a hot spot within the field that was surrounded by regions of lower sensitivity. Although no monocularly driven glial cells were found, some cells clearly responded better to one eye than to the other. In one instance we recorded from a glial cell that responded better to short vertical slits than to

long slits of any orientation. The responses of this cell may have been dominated by one or more hypercomplex cells in a particular orientation column. However, glial cells generally responded poorly to stationary stimuli, and we obtained no further clues as to whether glial cells tend to associate with particular functional groups of neurones.

DISCUSSION

Neurones

The spectrum of cells injected in this study included examples of most of the cell types found in Golgi preparations of area 17. The only cell types not seen were a few relatively scarce varieties such as horizontal cells and arachniform cells. A clear correlation was found between the main functional and morphological classes of neurones in the visual cortex. Eight of the ten injected stellate cells had simple receptive fields, while only three of the thirty-two pyramids were simple. The correspondence seems less striking when the results are examined in a different way, since only eight of the thirteen simple cells were stellate. In reality, though, the proportion of simple cells that are stellate may be greater than this because it is probably easier to stain pyramidal cells than stellate cells. This argument is supported by the results for layers IV*c* and V, two laminae of approximately the same thickness. Only three cells were stained in IV*c*, a zone rich in small stellate cells, whereas a total of twelve neurones, eight of which were large pyramids, were injected in layer V. Furthermore, we routinely found that simple cells were more difficult to impale and inject than complex cells.

The observation that both simple cells and stellate cells are concentrated in layer IV leads one to question whether it is the shape of a neurone or its position that is most important in determining its function: for example, are stellate cells outside of layer IV as likely to have simple receptive fields as those within layer IV? We do not have a large enough sample of injected cells to answer this question decisively. Only four stellate cells outside of layer IV were stained, and two of these were simple cells. Another way of approaching the same issue is to ask whether there is a correspondence between cell shape and function within individual cortical layers. In layer IV six of the seven simple cells, but none of the three complex cells, were classified as stellate. In layers V and VI all of the sixteen complex cells, but none of the three simple cells, were pyramidal. No such correlation was found for the sample of cells injected in layer II + III. The presence of a substantial population of hypercomplex cells in this layer means that the relationship between structural and functional classes cannot be exactly the same in all layers. Further

studies will be necessary to determine just how precise this relationship is and how it varies from one layer to the next. Our present results are consistent with the idea that the connexions made on to a cortical cell depend both upon its shape and its position.

We have made the distinction between morphological cell types largely on the basis of cell shape and dendritic geometry; this was because dendritic spines and axonal processes were not consistently stained in our injected cells. The division into primary cell classes can, however, be made on other grounds. For example, both Golgi (1886) and Cajal (1911) stressed that cortical neurones can be grouped into those with short, locally ramifying axons, and those with long axons leaving the cortex. This approach in a sense accentuates neuronal output rather than input, but of course the two are correlated given that most stellate cells have short axons and most pyramids have long axons. Another division exists between spine-bearing neurones and spine-free or sparsely spined cells. This may be quite important in view of recent evidence that spiny stellate cells are more similar to pyramids than to spine-free neurones with respect to the distribution of synaptic inputs on to their dendrites and the types of synapses made by their axons (Le Vay, 1973).

It is difficult to know *a priori* which of these anatomical classifications will turn out to be the most reliable in predicting the functional role of a cell. The fact that there is a strong correlation between the identification of a cell as stellate or pyramidal on the one hand and simple, complex, or hypercomplex on the other increases one's confidence that both of these classification schemes are based upon important principles of cortical organization. However, the structural features used for classifying cells, such as dendritic and axonal distributions, may be only indirect reflexions of the factors governing the specificity of connexions between neurones. With regard to the two subtypes of stellate cell, at least one of the injected simple cells was a spiny stellate neurone (Text-fig. 2*A*); yet many spine-free cells must also be simple since they are common in layer IV*c*, where there are virtually no complex or hypercomplex units. Thus at present the functional significance of the striking differences between spine-free and spiny stellate cells remains uncertain. We also have no real understanding of why there are so many unusual cell types, such as double bouquet cells and crescent cells.

Cells within area 17 display great diversity in their physiological properties, and it is natural to wonder to what extent the detailed structure of a cell reflects its particular functional properties. For example, cells projecting to the superior colliculus are concentrated in layer V and have characteristic physiological responses (Palmer *et al.* 1972); in our experiments cells with these properties were found to be pyramids (Table 3), thus

supporting the observations of Clark (1942) that tectal lesions cause degeneration of many layer V pyramids. However, aside from the correlation between the primary morphological and functional cell types, we found no obvious correlation between cell morphology and a variety of physiological properties such as directional selectivity, speed preference, and ocular dominance. This point can be illustrated by considering the relationship between the size of an injected cell and the size of its receptive field. Complex cells on the average have larger receptive fields than simple and hypercomplex cells and also tend to have larger cell bodies and dendritic arbors. Yet within each class of cells there is no close relationship between cell size and receptive field area. This demonstrates, not surprisingly, that even within a small region of the cortex there are other factors besides dendritic spread that influence the receptive field sizes of neurones.

All of the cortical neurones we stained showed clear orientation selectivity. The non-oriented cells we encountered had centre-surround receptive fields typical of geniculate afferents. One of these units was actually stained; it appeared to be an axonal process, presumably of geniculate origin.

The functional organization of the monkey striate cortex is similar in many respects to that of area 17 in the cat, except that many of the units in layer IV of the monkey have non-oriented receptive fields (Hubel & Wiesel, 1968). These cells probably represent a processing stage preceding that of oriented cortical units. It will be of interest to examine whether the insertion of a separate functional class of cells results in an altered relationship between structure and function for simple and complex cells.

Finally, it is worth while to consider how the present results relate to the suggestion that cortical cells are arranged in a hierarchy of increasing complexity, from simple to complex to hypercomplex (Hubel & Wiesel, 1962, 1965). Our findings are certainly consistent with this idea but they provide only one link in the chain of evidence needed to provide a convincing demonstration of its validity. The evidence that most stellate cells in layer IV are simple sets the stage for testing this hypothesis by determining anatomically whether the geniculate afferents arriving in layer IV end selectively or preferentially on stellate neurones. It is already known that geniculate fibres terminate predominantly on dendritic spines (Garey & Powell, 1971), but this of course does not favour pyramids over stellate cells as the terminal sites, nor does it rule out the possibility that some afferents end on spine-free or sparsely spined cells.

Glial cells

Previous attempts to identify glial cells in the cerebral cortex by injecting either methyl blue or fast green have been only partially successful because of the difficulty in staining cells completely (Kelly, Krnjević & Yim, 1967; Grossman & Hampton, 1968; Sugaya *et al.* 1971). We were able to inject and identify seven glial cells (six astrocytes and one oligodendrocyte), but the injections were not entirely satisfactory because the cells were only faintly stained. Part of our confidence that electrically inexcitable cells in the cortex are indeed glia rests upon the similarity of their physiological properties to identified glial cells in other preparations (Kuffler & Potter, 1964; Kuffler *et al.* 1966; Dennis & Gerschenfeld, 1969).

Glial cells in area 17 can respond to visual stimuli with graded depolarizations up to 7 mV in amplitude. These responses are similar in size and time course to the glial depolarizations studied in a variety of preparations, including the mammalian cerebral cortex (Orkand *et al.* 1966; Karahashi & Goldring, 1966; Baylor & Nicholls, 1969; Ransom & Goldring, 1973). Glial depolarizations have been shown to result from an increase in extracellular K concentration following impulse activity in surrounding neurones; one can therefore assume that glial cells simply detect any neuronal activity in their immediate environment. The preference of some glial cells in area 17 for stimuli of a particular orientation presumably reflects the organization of the cortex into orientation columns (Hubel & Wiesel, 1962). However, most responsive glial cells gave at least some depolarization to stimuli of any orientation and were not as sharply tuned as neurones for orientation, There are several possible explanations for this broad orientation selectivity. First, glial cells that are near the borders of an orientation column may sample the activity of neurones in adjacent columns. Second, part of the glial response may be contributed by K release from non-oriented geniculate afferents and from synaptic activity that is more broadly tuned than the resultant neuronal output. Finally, glial cells may be electrically coupled to their neighbours, so that a depolarization in one cell could reflect contributions from glia in nearby orientation columns. Although we have no direct evidence on this issue, the last suggestion seems likely to be an important factor. Glial cells in leech ganglia and in the optic nerves of amphibia are electrically coupled (Kuffler & Potter, 1964; Kuffler *et al.* 1966), and gap junctions exist between glia in the cerebral cortex (Brightman & Reese, 1969). If cortical glia are coupled by low resistance junctions it would help to explain why stable penetrations with high resting potentials can be obtained from cells that are so small.

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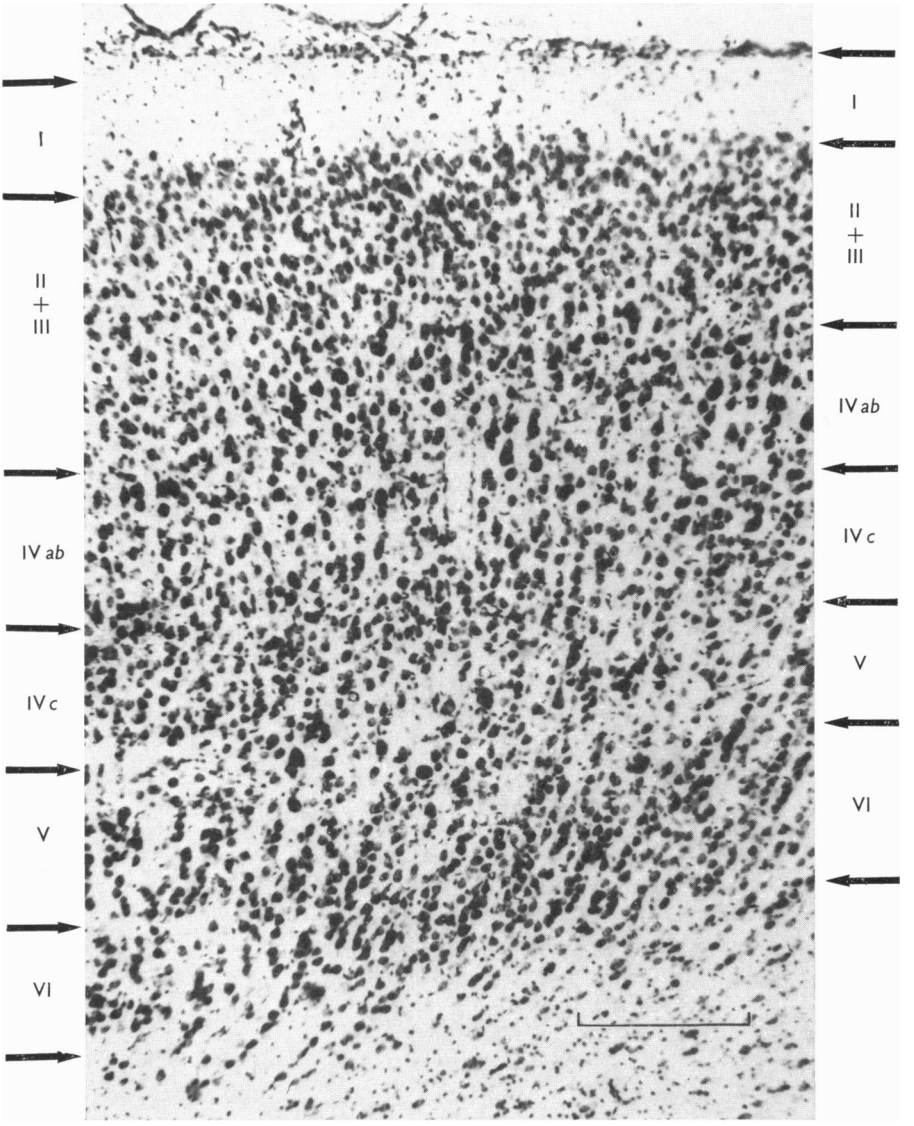
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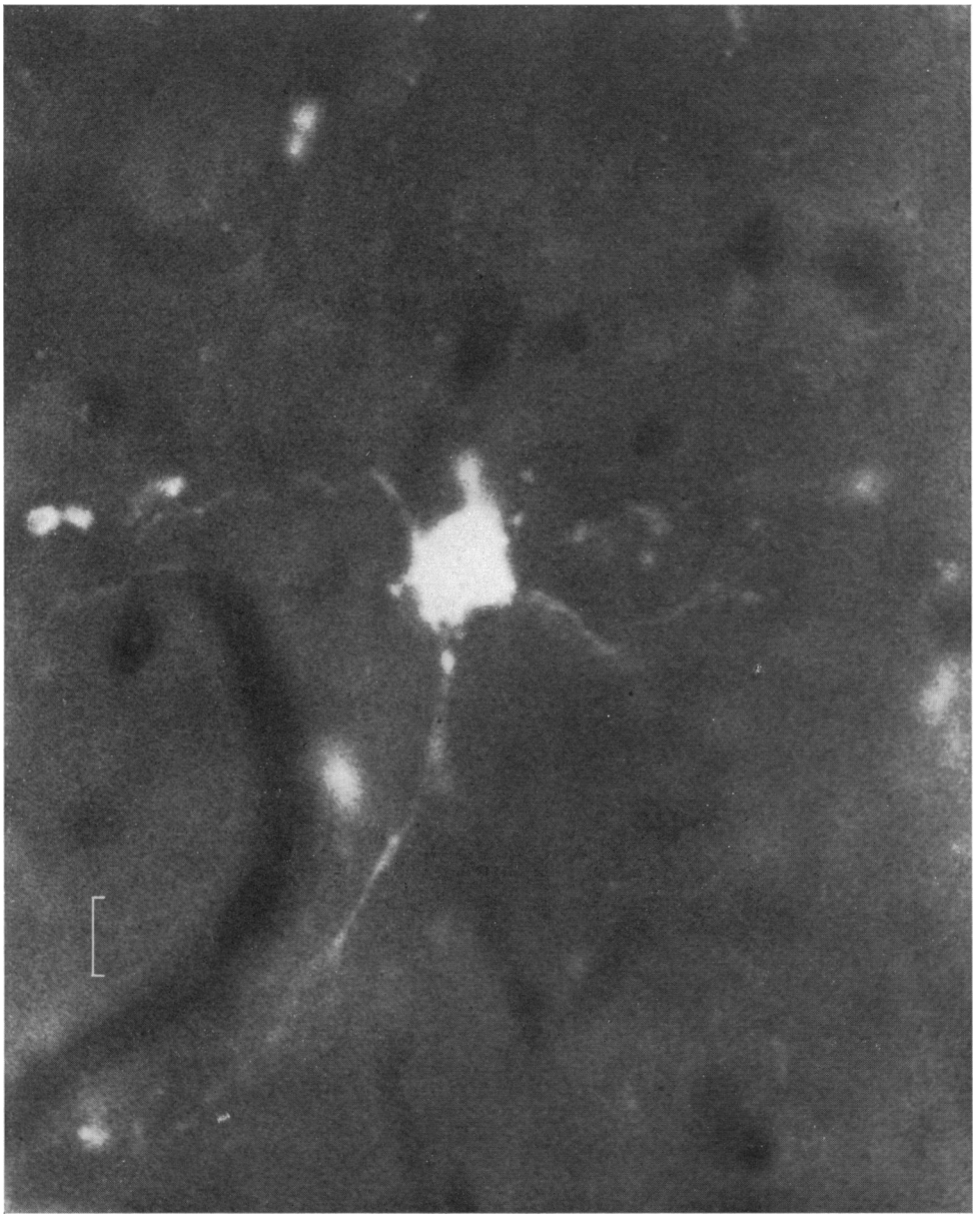

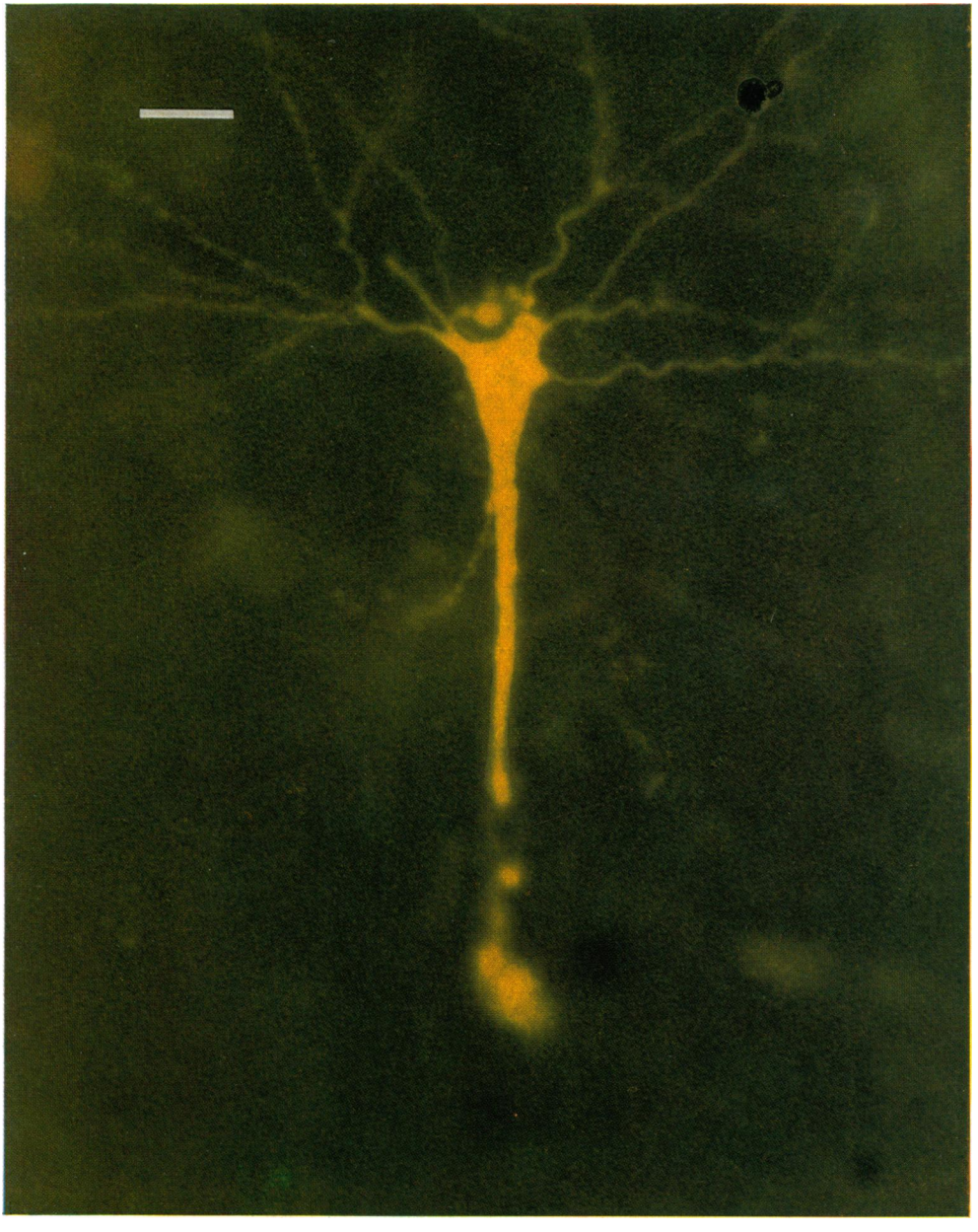

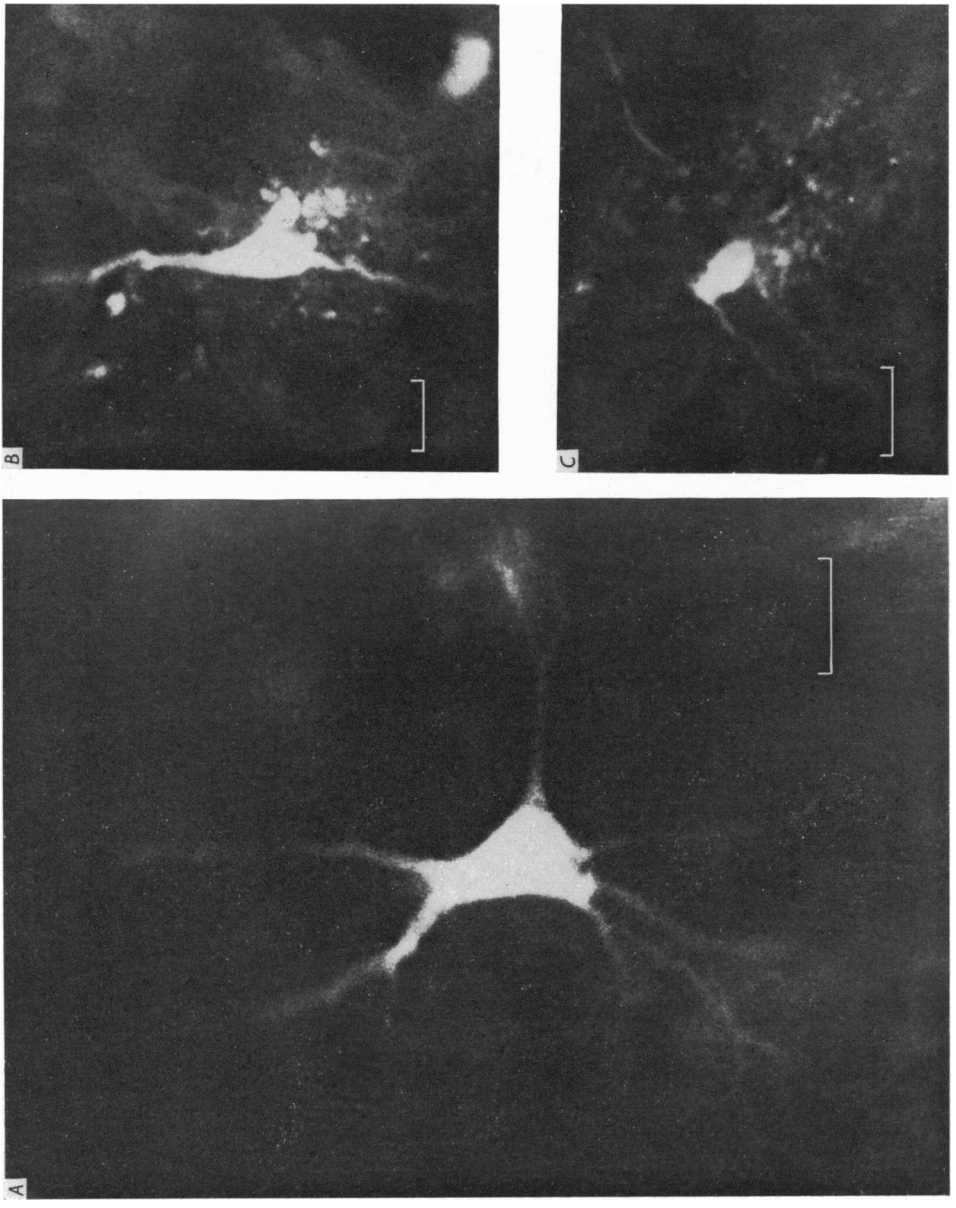
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EXPLANATION OF PLATES

PLATE 1

Photograph of a Nissl-stained section of area 17 in the cat, near the apex of the post-lateral gyrus. The layering nomenclature is adopted from Otsuka & Hassler (1962). Each layer turns upward, from lower left to upper right, as it runs towards the apex. Unlike the primate cortex, most of the boundaries between the layers are rather vague. The calibration bar is 200 μm.

PLATE 2

An injected simple stellate cell from layer IV*ab*. Most of the processes of this cell are out of focus owing to the thickness of the section (80 μm), but the outline of the cell body can be seen. A camera lucida drawing of this cell is presented in Text-fig. 2*A*, which gives a better representation of its stellate shape. The calibration bar is 10 μm.

PLATE 3

A photomicrograph of an injected complex cell from layer V. This cell is clearly a pyramid even though the full extent of the apical dendrite is not visible. Several of the basal dendrites displayed spines that are not clearly visible in this photograph. The calibration mark is 10 μm.

PLATE 4

Photographs of an injected hypercomplex cell and two injected glial cells. *A*, a pyramidal hypercomplex cell with an apical dendrite that bifurcates just above the cell body. The physiological properties of this cell are described in Table 4. The calibration mark in *A* is 20 μm. *B*, a glial cell injected in Layer V and tentatively identified as an oligodendrocyte. It did not respond to visual stimuli. *C*, a glial cell injected in Layer II + III that was identified as an astrocyte; its physiological responses are shown in Text-fig. 14*A*. The calibration marks in both *B* and *C* are 10 μm.