

Morphology of physiologically identified neurons in the visual cortex of the cat

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Most cells in cortical area 17 of the cat can be physiologically classified as simple or complex^{3,13,16}. Both types of cells are found virtually in every layer, but simple cells are concentrated in layer IV, while complex cells are more frequently recorded in layer V^{2,3,8}. Anatomical studies indicate that stellate cells occur most frequently in layer IV, and pyramidal cells are found in other layers^{9,10,14}. Thus, the stellate cells may have simple receptive fields; and pyramidal cells, complex receptive fields.

Kelly and Van Essen related neuronal structure to function with a more direct technique⁵. They impaled physiologically identified cells and filled them with the dye, Procion yellow. From this, they concluded that most complex cells are morphologically pyramidal, whereas most simple cells are not. We re-investigated this problem using horseradish peroxidase (HRP), instead of a fluorescent dye, to stain the cell^{1,4,6}. This seemed to give a more complete morphological picture than can generally be obtained with Procion dyes. Our preliminary observations generally support those of Kelly and Van Essen⁵, but some details have been added.

Normal adult cats were used for these studies. The physiological preparation, anesthesia, and recording techniques have been described previously^{1,13,16}, with minor exceptions noted as follows. Micropipettes were filled with 2–3% HRP in 0.2 M KCl, buffered with 0.05 M Tris at pH 8.6, and their tips were bevelled (100–200 M Ω at 200 Hz; o.d. < 0.5 μ m). The electrode was inserted vertically into the medial wall of area 17. Cells were isolated extracellularly and classified as simple or complex^{3,13,16}. We then advanced the electrode in 1 μ m steps to impale the neuron. Intracellular recording was verified by previously published criteria (ref. 1; see also Fig. 1A, C of this report), and we re-examined the physiological properties to verify that the cell was the same as classified extracellularly. Next, we injected HRP into the cell by

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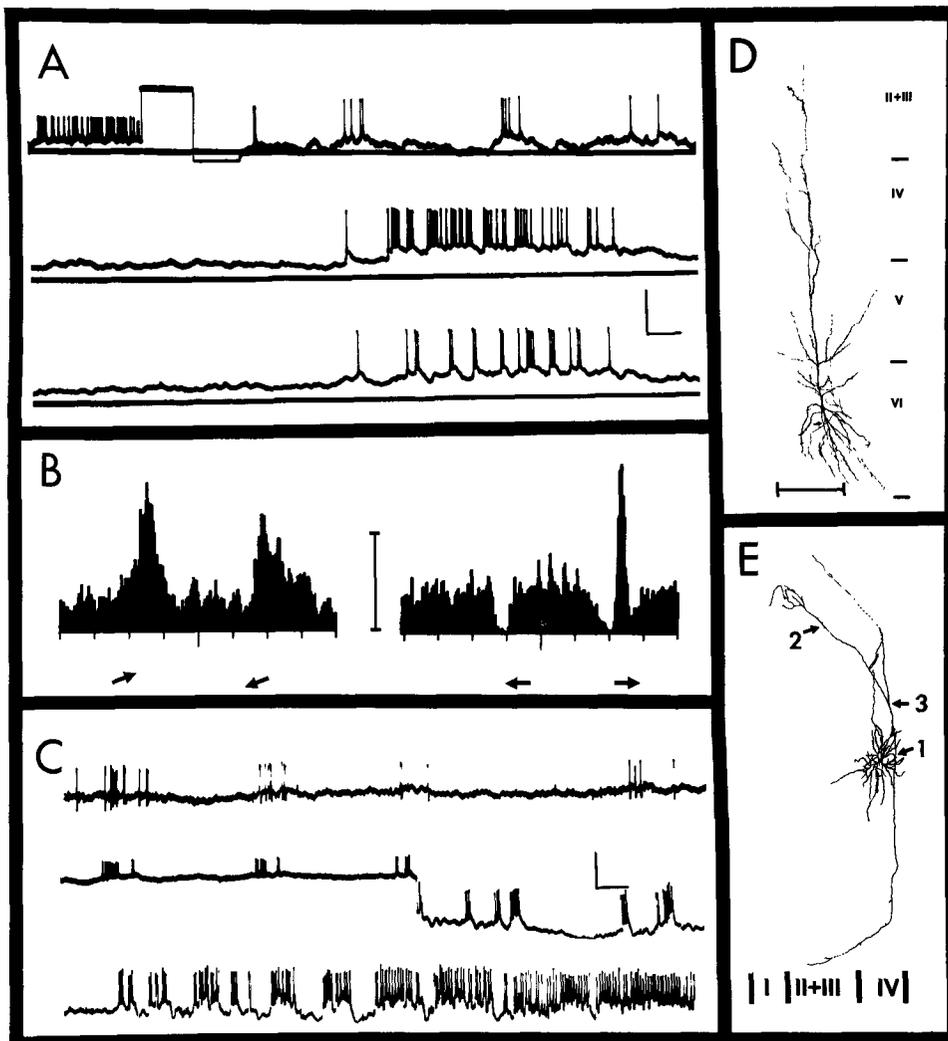


Fig. 1. Representative physiological and morphological data from area 17 complex and simple cell injected with HRP. A: records from the complex cell. The top record shows the initial extracellular recording followed by a depolarizing current pulse which caused impalement of the cell. The ensuing 65 mV drop in membrane potential moved the record off scale, so the DC level was repositioned. The thick, horizontal line through these records is a reference trace against which it is easier to appreciate slow polarization changes. The intracellular spikes ride upon slow depolarizing waves. The middle and bottom records represent intracellular responses to a bright bar swept across the receptive field at 5°/sec. Again, note that the spikes ride upon depolarizing waves, and spike bursts are interrupted by small hyperpolarizing potentials. Scale: 100 msec and 50 mV. B: peristimulus histograms from the complex (left) and simple cell (right). These relate neuronal firing rate to the position of a bright bar moved at 5°/sec across the receptive field. The left half of each histogram represents movement of the stimulus in one direction, the right half, in the reverse direction. (The arrows indicate the stimulus direction.) The complex cell's histogram was obtained during intracellular recording and typifies histograms for such cells^{13,16}. The simple cell's histogram, obtained during extracellular recording, displays the suppressive zones flanking the narrow discharge center, and this is typical of simple cells^{13,16}. This simple cell, like most, had a very low spontaneous rate; its background activity was artificially elevated by monocular conditioning^{13,16}. Scale: horizontal divisions, 1.5° of visual field; vertical bar, 120 spikes/sec for left histogram, and 80 spikes/sec for right histogram. C: records from the simple cell. The top trace is an extracellular record taken while a visual stimulus was swept through the receptive field. The middle trace is a lower gain record that shows the transition from 'quasi-intracellular' to true intracellular recording, indicated by the large drop in the DC potential, plus the large spike amplitude and slow wave activity. The bottom trace shows intracellular recording 5 min following completion of the iontophoretic injection of HRP. The high discharge rate, seen in occasional cells after such injection, suggests membrane damage. Scale: 100 msec for all traces and 2 mV (top trace) or 50 mV (middle and bottom trace). D: line drawing of the complex cell. It has pyramidal morphology with dendritic spines. The arrow indicates the axon, and the scale is 100 μ m. This scale also serves as a 200 μ m marker for E. E: line drawing of the simple cell. The numbered arrows point to axonal regions illustrated in the photomicrographs of Fig. 2C, D, F.

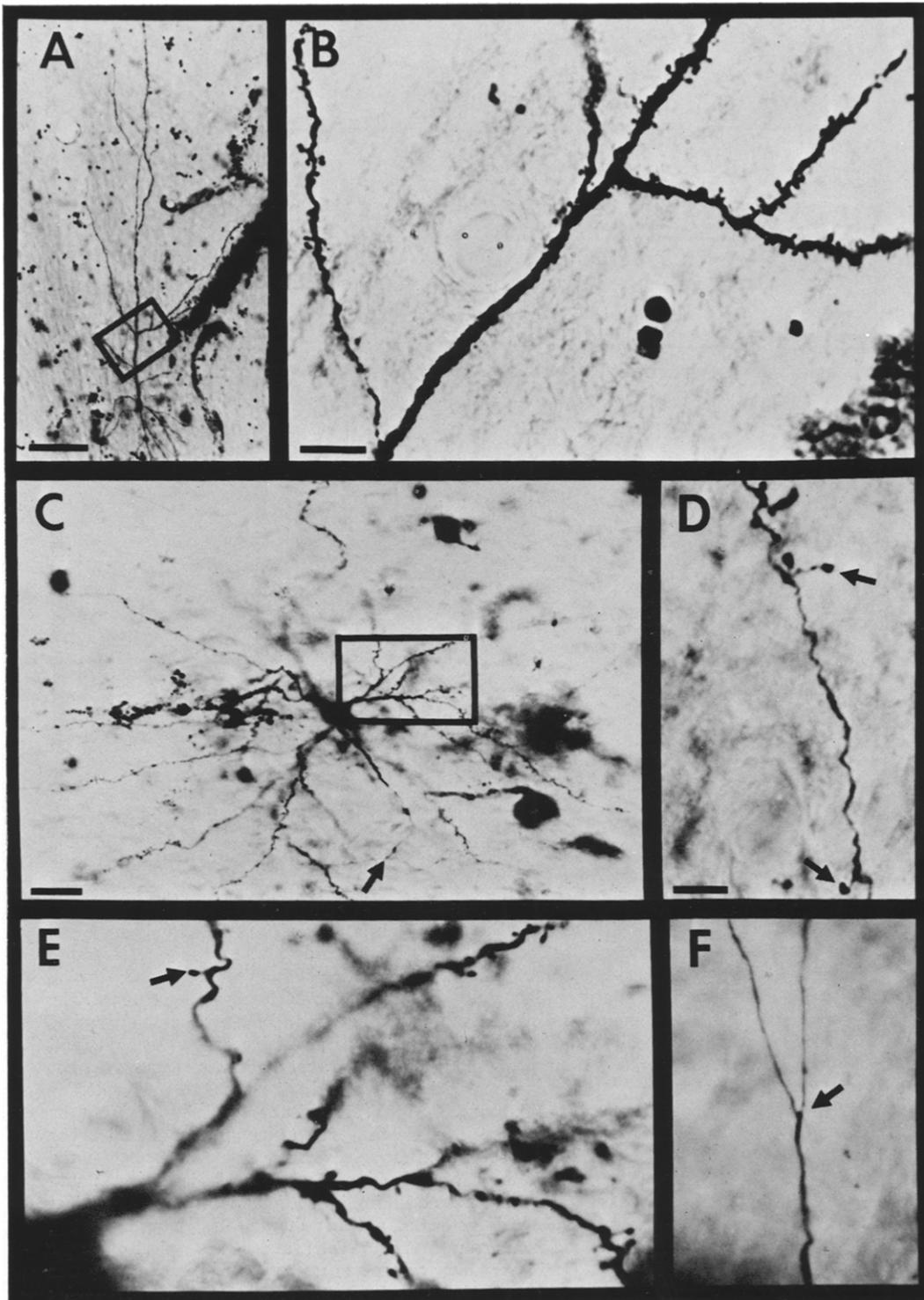


Fig. 2. Photomicrographs of complex cell (A, B) and simple cell (C-F). Physiological records and line drawings of these same cells are shown in Fig. 1. The pial surface in A and C is towards the top of the figure. A: pyramidal cell with soma in layer VI. This cell had a complex receptive field. The rectangle defines the field of view in B. Scale: $100\ \mu\text{m}$. B: dendrites, with obvious spines, of cell shown in A. Scale: $10\ \mu\text{m}$. C: Spiny stellate cell with soma in layer IV. This cell had a simple receptive field. The arrow points to the axon's first branch point, from which collaterals run large distances parallel to the layering. This corresponds to arrow 1 in Fig. 1E. The panel outlines the field of view in E. Scale: $50\ \mu\text{m}$. D: view of axon of the simple cell from region corresponding to arrow 2 in Fig. 1E. The arrows here indicate appendages which may be presynaptic terminals. Scale: $10\ \mu\text{m}$ and applies as well to E, F. E: dendrites of simple cell with numerous spines. One of these is indicated by the arrow. F: region of axon of simple cell indicated by arrow 3 in Fig. 1E. The bifurcation clearly seen here is emphasized by the arrow.

iontophoresis (5 nA positive pulses of 200 msec duration at 3 Hz for 3–5 min). Normal electrical activity was typically seen in the cell during iontophoresis, and we were thus certain that intracellular recording was maintained during the HRP injection. Occasionally, intracellular recording was maintained ≥ 45 min. This allowed acquisition of response histograms during intracellular recording (Fig. 1B). We injected ≤ 4 cells per hemisphere, each cell spaced > 1.5 mm apart. After 2–24 h following the intracellular HRP injections, the cats were re-anesthetized and perfused intracardially with buffered 1% glutaraldehyde and 1% paraformaldehyde. Frozen sections were cut coronally at $120 \mu\text{m}$ and reacted with diaminobenzidine⁷. Sections adjacent to HRP-filled cells were counterstained with cresyl violet to determine laminar relationships. We drew labelled cells through a $100 \times$ oil immersion objective (NA 1.32) by means of a microscope drawing tube. During drawing and photography, we usually employed a Kodak Wratten 48A or 49B filter to enhance contrast (see Fig. 2)¹.

Fig. 1A–C shows representative physiological data from a complex cell and a simple cell. Photomicrographs from the same two neurons are illustrated in Fig. 2. They were intracellularly injected with HRP, and considerable morphological detail is evident. Line drawings of these two cells are shown in Fig. 1D, E. The complex cell has pyramidal morphology, while the simple cell has stellate morphology. The axon branches of the simple cell spread from the soma in both directions parallel to the pial surface for over 1 mm before turning dorsally and terminating in layers II and III. This extent greatly exceeds the reported dimensions of ocular dominance or orientation columns^{3,12}.

To date, 14 cells (5 complex, 9 simple) from area 17 have been classified both morphologically and physiologically. Each of the 5 complex cells exhibits pyramidal structure with a soma in layer V or VI. The simple cells are more heterogeneous morphologically. They include one non-spiny stellate cell with a soma in layer II or III; one spiny stellate cell with a soma in layer IV (shown in Figs. 1 and 2); one crescent-shaped cell with a soma in layer V; and 6 pyramidal cells with somata in lower layer III (one cell), layer V (one cell), or layer VI (4 cells). We have not yet found obvious morphological differences between the simple and complex pyramidal cells.

Several conclusions can be drawn from these preliminary results: (a) Intracellular HRP filling of cells permits a reliable, complete, and detailed structural analysis of the cells and their processes. Axons were often traced several mm into white matter, and frequent axon collaterals with terminal swellings were evident in gray matter. (b) Each of our complex cells had pyramidal morphology, and each of the stellate cells was simple⁵. (c) However, several simple cells had pyramidal morphology. Kelly and Van Essen⁵ found only 3 of 31 simple cells with pyramidal morphology (as opposed to our 6 of 9), and none of their simple pyramids had a soma in infragranular layers (5 of our 6 did).

These results suggest 3 possibilities that are currently under investigation. First, the heterogeneity of simple cell morphology may underlie a hitherto unseen physiological heterogeneity². The finding that all complex cells were pyramidal suggests the relative functional homogeneity of this group, but our sample is still quite small. Second, a more detailed analysis might uncover structural differences in the simple and

complex subgroups of pyramidal cells. Third and most significant, perhaps our failure to find a perfect correlation between receptive field type and the pyramidal/non-pyramidal morphological type results from irrelevant classification schemes. For instance, perhaps functional parameters providing cell classes orthogonal to the simple/complex classification^{11,15} are more meaningful for structural correlates. Likewise, the gross morphological appearance of pyramidal and stellate cells could be unrelated to subtler structural parameters which underlie the simple complex functional differences.

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