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Structure/function relationships of retinal ganglion cells in the cat

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Intracellular recording and horseradish peroxidase (HRP) iontophoresis was used to define structure/function relationships for single retinal ganglion cells in the intact cat eye. Fifteen physiologically characterized cells were labeled as follows. Five W-cells had gamma morphology, 6 X-cells had beta morphology, and 1 Y-cell had alpha morphology, and these relationships support earlier conclusions. However, one cell could not be physiologically classified despite beta morphology, one X-cell was not a beta cell, and one Y-cell was not an alpha cell. Whether these unusual structure/function relationships represent an artifact of methodology or complications to be added to prevailing notions requires further study.

The cat's retinofugal projection consists of at least 3 parallel and functionally independent pathways that begin with physiologically distinct classes of retinal ganglion cells (for reviews, see refs. 10, 14, 15, 20). These have been called W-cells (or sluggish-sustained and sluggish-transient cells), X-cells (or brisk-sustained cells), and Y-cells (or brisk-transient cells). Three major morphological classes, called alpha cells, beta cells, and gamma cells, have also been described for these neurons[2]. Data from several studies, most notably those of Wässle and his colleagues[2,4,9,12], suggest that Y-cells are alpha cells, X-cells are beta cells, and W-cells are gamma cells (see also refs. 6, 11, 19). However, except for the alpha/Y-cell correlation, the methods used to establish these relationships have been indirect and somewhat uncertain. We applied the technique of intracellular staining of ganglion cells with horseradish peroxidase (HRP) to characterize directly the morphology of physiologically identified W-, X-, and Y-cells[5,16]. Furthermore, we recorded these neurons in an intact, in vivo preparation in order to obtain response properties more comparable with those previously reported for retinal W-, X-, and Y-cells. Our initial and preliminary results confirm most of the structure/function relationships proposed by Wassle and his colleagues[2,4,9,12], although certain unexpected relationships were also seen.

The general methods used in this study are similar in nearly all respects to those we have previously reported for analogous studies of geniculate W-, X-, and Y-cells[5,16]. Adult cats were anesthetized, placed in a stereotaxic headholder, paralyzed, and artificially ventilated. We stabilized the left eye (from which all data were collected) by cementing its conjunctiva to a ring behind the limbus. The pupil was dilated with atropine and the cornea covered with a contact lens chosen by retinoscopy to focus the eye on visual stimuli. A recording and injection micropipette (filled with 3–5% HRP, 0.2 M KCl, and 0.05 M Tris buffer; bevelled to 80–110 MΩ) was placed transclerally into the retina through a guide needle. A specially designed manipulator permitted movement of the electrode tip across the retinal surface under ophthalmoscopic control. Bipolar stimulating electrodes were placed to straddle the optic chiasm and both optic tracts just below the lateral geniculate nuclei. Receptive fields of single cells were analyzed by means of visual stimuli that consisted of bright and dark spots and annuli projected onto a frontal tangent screen as well as sinusoidally counterphased, sine wave gratings generated on a cathode ray tube

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placed in front of the eye. The gratings had a mean luminance of 38 cd/m^2 and continuously variable spatial frequency, temporal frequency, and contrast. The technique described by Pettigrew et al.[13] was used to plot the retinal blood vessel pattern onto the same tangent screen used for plotting receptive fields. The position of blood vessels was re-checked after each receptive field was plotted.

Each ganglion cell was first recorded extracellularly, and a number of response properties were studied. These include: (1) latency to antidromic (electrical) stimulation of the optic chiasm and tract; (2) receptive field location and size; (3) briskness of response to visual stimuli; (4) linearity of spatial and temporal summation; and (5) nature of the center/surround receptive field organization, if present. Ganglion cells were identified as W-, X-, or Y-cells on the basis of the following previously described criteria[3,7,18]. Compared to X- and Y-cells, W-cells tend to have larger receptive field centers, longer latencies to antidromic stimulation, and more sluggish responses. Also, tonic on center W-cells do not respond to a dark spot moving out of the receptive field center, and the same is true for tonic off center W-cells and bright spots; this is the 'centrifugal test' described by Cleland and Levick[3] (see also ref. 18). X-and Y-cells respond vigorously on this test. Finally, compared to X-cells, Y-cells tend to exhibit larger receptive field centers, shorter latencies to antidromic activation, less linear spatial and temporal summation, and more phasic responses to standing contrasts. After identification of the ganglion cell, the electrode penetrated the neuron, and the cell was quickly re-classified during intracellular recording. Depolarizing pulses forced HRP into the cell[5,16]. The electrode was withdrawn from the retina, and a new retinal recording site was chosen for another cell at least 5° from any previously injected neuron. At the conclusion of the recording session, the cat was deeply anesthetized and perfused transcardially with aldehyde fixatives. We then dissected the retina, reacted it with 3,3′-diaminobenzidine plus cobaltous chlo-

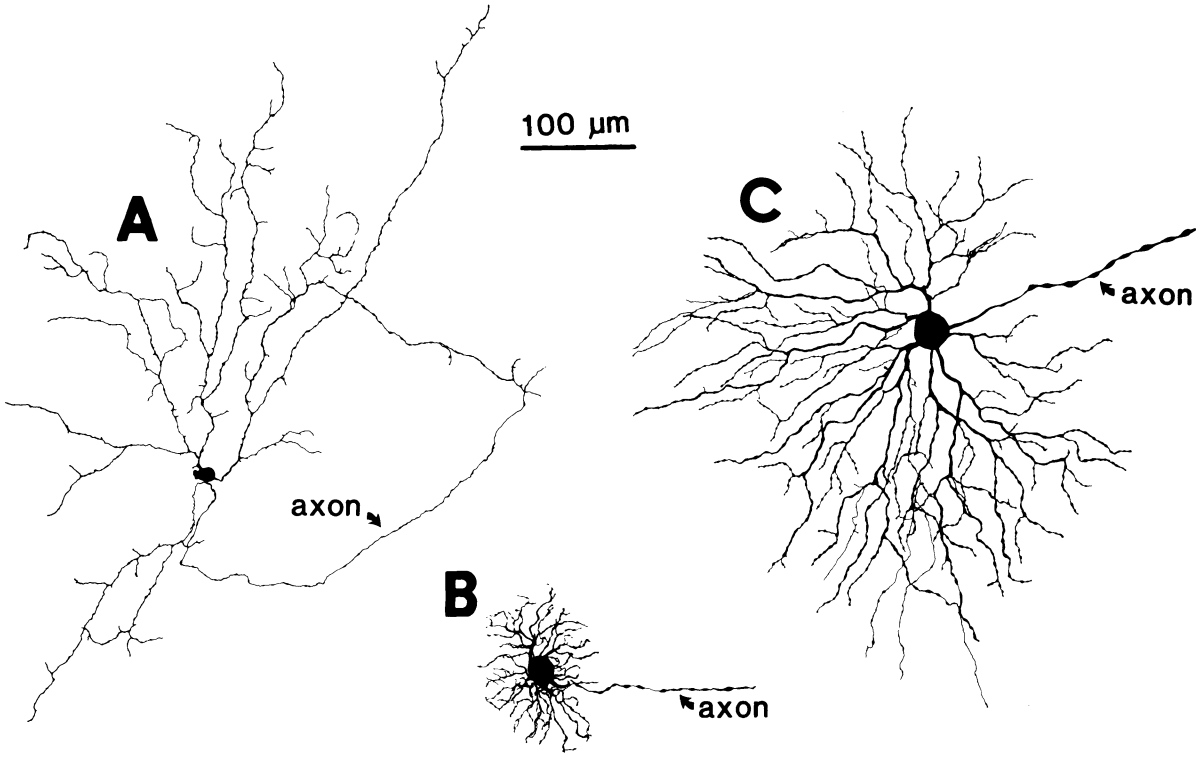

Fig. 1. Tracings of typical ganglion cells in flat-mounted retinas. A: W-cell located 36.5° nasal to and 7.5° below the area centralis (in retinal coordinates). The cell exhibited linear summation to grating stimuli, had an off center, and was antidromically driven from the optic chiasm with a latency of 5.0 msec. B: X-cell located 3.5° temporal to and 9.0° below the area centralis. The cell had an off center and was antidromically activated from the optic chiasm at a latency of 3.8 msec. C: Y-cell located 1.5° nasal to and 5.0° below the area centralis. It had an off center and was antidromically activated from the optic chiasm with a latency of 1.6 msec.

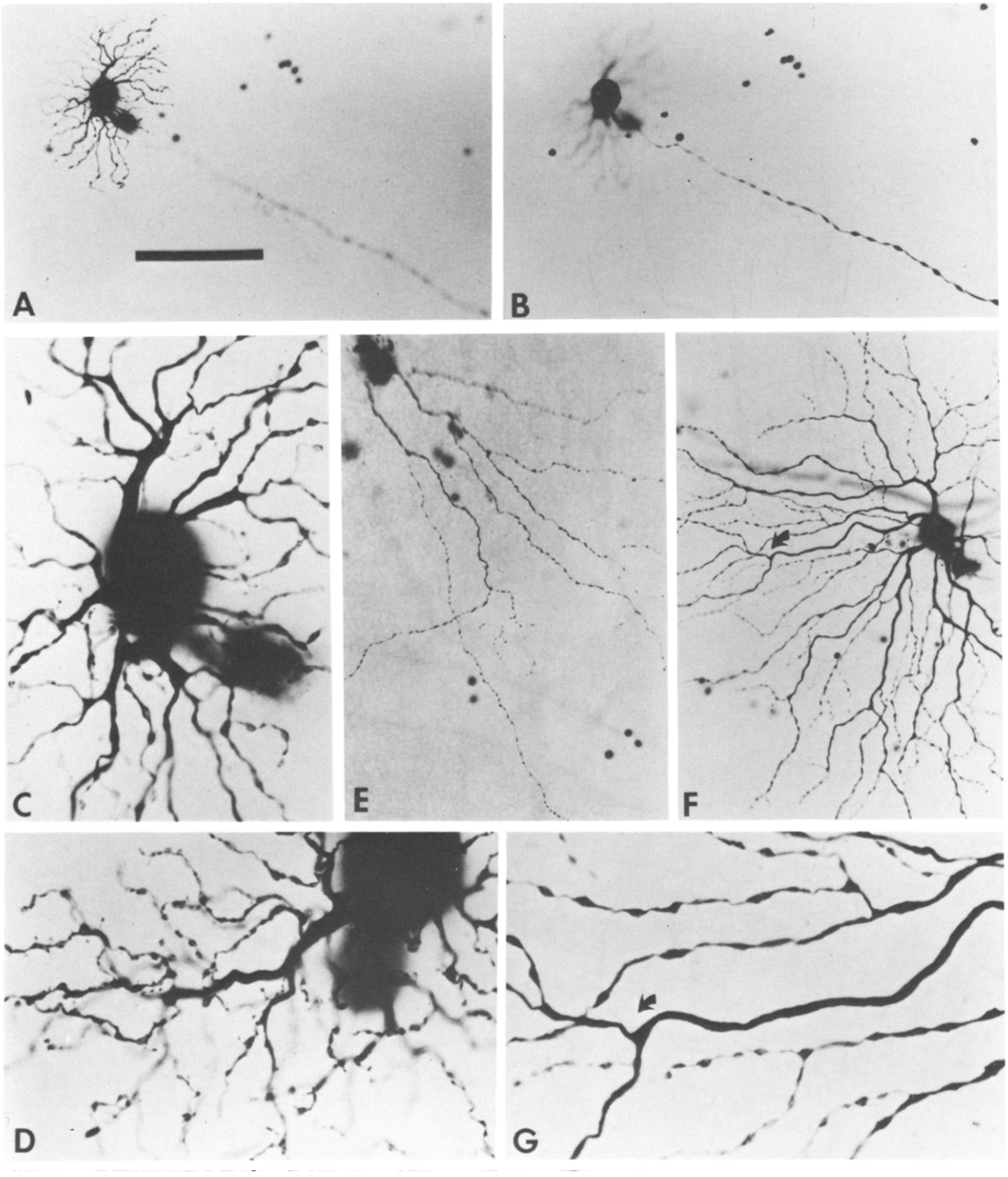

Fig. 2. Photomicrographs of HRP-filled retinal ganglion cells in flat-mounted retinas. The scale in A is 100 μm for A, B, E, and F, and is 25 μm for C, D, and G. A and B: Two focal planes showing the dendrites (A) and soma plus axon (B) of the same X-cell drawn in Fig. 1B. C: higher power view of dendrites of cell illustrated in A, B. D: dendrites of another X-cell with beta morphology. E: same W-cell as drawn in Fig. 1A. F and G: same Y-cell as drawn in Fig. 1C in lower (F) and higher (G) power. For orientation, the arrows in F and G indicate the same dendritic branch point.

ride[1], and flat-mounted it on a slide. The position of recovered neurons relative to blood vessels always matched the position of its receptive field relative to the projected blood vessel pattern seen during recording.

We have thus far injected and recovered 15 retinal ganglion cells, all of which had center/surround receptive field organization. With one exception noted below, each of these represented the only labeled cell in the region. These include 5 W-cells, 7 X-cells, 2 Y-cells, and one unclassified cell. Figs. 1 and 2 show typical examples of a W-cell that was a gamma cell, an X-cell that was a beta cell, and a Y-cell that was an alpha cell. Most of the labeled cells had varicose dendrites, and most also had curiously varicose axons that could be traced into the optic disc. It is not clear whether these varicosities are some artifact of the HRP filling or whether they represent a functionally significant morphological feature. Some of the cells had numerous, fine processes appended to their dendrites (e.g. Fig. 2D). Also, as exemplified by Figs. 1 and 4, most of the somata were eccentrically placed within the dendritic arbor.

Each of the 5 W-cells was a typical gamma cell with a sparse, diffuse dendritic arbor and relatively small soma. Fig. 3, which illustrates the soma size distributions of the recovered cells, shows that the W-cells had smaller somata than every X-cell.

Six of the 7 X-cells exhibited typical beta cell morphology with medium-sized somata and dense, restricted dendritic arbors. The seventh X-cell had unusual morphology. Although its soma fell within the size range of beta X-cells (arrow in Fig. 3), its dendritic arbor was sparser than those of beta cells and more restricted and denser than those of gamma cells (Fig. 4A). This cell's morphology is similar to types previously described (e.g., the delta cell[2] and medium-sized gamma cells[17]) that were thought to be W-cells.

One of the Y-cells appeared to be a typical alpha cell (Figs. 1C and 2F). This is the one example for which another labeled cell was also seen nearby. Fig. 2F shows another cell body with processes below and to the right of the alpha cell body, and this other cell was not included in the drawing of Fig. 1C. However, this other cell was located in the inner plexiform layer as judged by its focal plane, had no axon, and was thus considered not to be the ganglion cell we recorded. It is not clear how this cell obtained its HRP label, but we had other examples of labeled

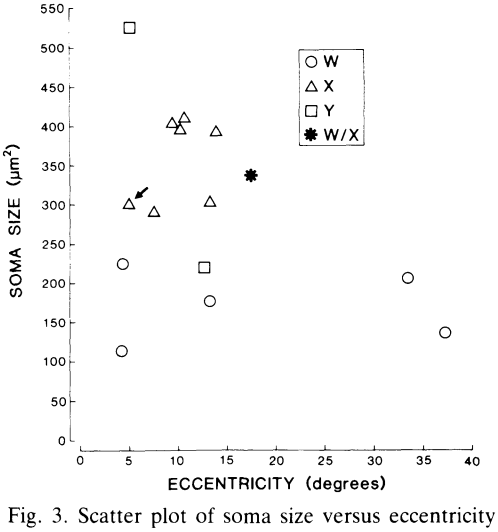

Fig. 3. Scatter plot of soma size versus eccentricity from the area centralis for identified W-, X-, and Y-cells plus one unclassified cell with properties of both W- and X-cells. The arrow indicates the X-cell illustrated in Fig. 4A.

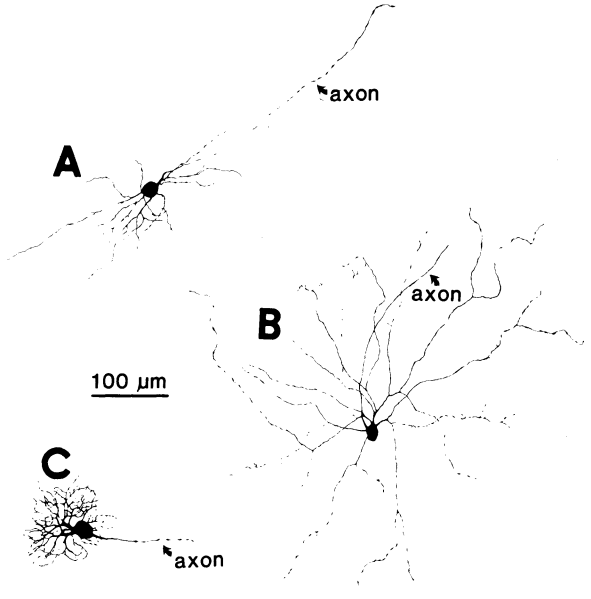

Fig. 4. Three retinal ganglion cells with unexpected morphology and/or response properties. A: X-cell with other than beta morphology. The cell was located on the vertical meridian 5.0° below the area centralis. It had a 1° diameter, tonic, off center receptive field and was antidromically activated from the optic chiasm with a latency of 2.8 msec. B: Y-cell with other than alpha morphology. The cell was located 4.5° nasal to and 12.0° below the area centralis. It had an on center and was antidromically activated from the optic chiasm with a latency of 1.6 msec. C: physiologically unclassified cell with beta morphology (see text for details).

doublets which are not included in our structure/function analysis. Our sole example of a Y-cell with alpha morphology is thus qualified. The other Y-cell had an unusually small soma, smaller indeed than every X-cell soma, and its dendritic arbor was not typical of alpha cells (Figs. 3 and 4B). It is quite possible that Y-cells with other than alpha morphology are exceedingly rare. Certainly our small sample should not be viewed as a serious challenge to the notion that the vast majority of Y-cells are alpha cells, although some are not.

Finally, one physiologically unclassified cell was a typical beta cell (asterisk in Figs. 3, and 4C). It exhibited an off center, tonic response to dark spots in the center, and linear spatial and temporal summation. Its latency to chiasm stimulation (5.3 msec) fell within the overlap region between W- and X-cells for its 7.5 mm distance from the optic disc[18]. Likewise, it seemed somewhat more sluggish than most other X-cells, but brisker than W-cells. However, while its 0.6° receptive field center diameter seemed too small for W-cells at an eccentricity of 17.5° (ref. 18), its failure to respond to the centrifugal test is characteristic of tonic W-cells[3,18]. Its beta cell morphology suggests the possibility that it was an X-cell and that the centrifugal test, at least as applied by us, may not always distinguish W-cells from X-cells.

Our findings support the hypothesis that each of the retinal W-, X-, and Y-cell classes has distinctive morphology. In the context of other data from our laboratory[5,8,16,21], each of the W-, X-, and Y-cell pathways from retina through the lateral geniculate nucleus to the visual cortex maintains unique morphological features. Also, although our results generally support the hypothesis that W-cells are gamma cells, X-cells are beta cells, and Y-cells are alpha cells, a number of qualifications still exist. Fig. 4 illustrates 3 cells out of our sample of 15 that suggest the need for more data. Apparently, not every cell identified as an X- or Y-cell has beta or alpha morphology, respectively (Fig. 4A and B). Also, either some cells with beta morphology are not X-cells or present physiological tests to distinguish W- from X-cells are inadequate (Fig. 4C). Since small Y-cells have not been described previously, they may represent an exceedingly rare type that we fortuitously sampled; conversely, they may represent a substantial fraction of Y-cells hitherto ignored in surveys of Nissl stained ganglion cells (e.g. refs. 4, 6, 22). Perhaps, as may be suggested by Fig. 2F, we did not always label the cell we recorded, and these unusual structure/function relationships represent a methodological artifact. Nonetheless, some questions regarding these relationships for retinal ganglion cells remain to be answered.

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Note added in proof

Since the preparation of this manuscript another physiologically-identified Y-cell with typical α cell morphology has been recovered. This neuron, like that illustrated in Fig. 2F, G, had a small, axonless cell associated with its dendritic field.