THE JOURNAL OF COMPARATIVE NEUROLOGY 396:84–104 (1998)

Functionally and Anatomically Segregated Visual Pathways in the Lobula Complex of a Calliphorid Fly

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ABSTRACT

In dipteran insects, the lobula plate neuropil provides a major efferent supply to the premotor descending neurons that control stabilized flight. The lobula plate itself is supplied by two major parallel retinotopic pathways from the medulla: small-field, magnocellular afferents that are implicated in achromatic motion processing and Y cells that connect the medulla with both the lobula plate and the lobula. A third pathway from the medulla involves transmedullary (Tm) neurons, which provide inputs to palisades of small-field neurons in the lobula. Although, in their passage to the brain, many output neurons from the lobula plate are separated physically from their counterparts in the lobula, there is an additional class of lobula complex output neurons. This group is composed of retinotopic lobula plate-lobula (LPL) and lobula-lobula plate (LLP) cells, each of which has dendrites in both the lobula and the lobula plate. The present account describes the anatomy and physiology of exemplars of LPL and LLP neurons, a wide-field tangential neuron that is intrinsic to the lobula complex, and representatives of the Tm- and Y-cell pathways. We demonstrate novel features of the lobula plate, which previously has been known as a motion-collating neuropil, and now also can be recognized as supporting direction- or nondirection-specific responses to local motion, encoding of contrast frequency, and processing of local structural features of the visual panorama. J. Comp. Neurol. 396:84–104, 1998. © 1998 Wiley-Liss, Inc.

Indexing terms: insect visual system; small receptive field efferents; parallel visual pathways; confocal microscopy; *Phaenicia sericata*

It is established that, in the cyclorraphan visual system (Fig. 1), the lobula plate collates directional motion information. Retinotopic inputs to the lobula plate, such as the type 5 bushy T cells (T5; Buschbeck and Strausfeld, 1996), carry directionally sensitive information from local groups of elementary motion-detecting circuits (EMDs; Douglass and Strausfeld, 1995). T5 neurons supply wide-field tangential neurons that are likewise sensitive to the direction of motion and appear to be relatively insensitive to simple flicker (Douglass and Strausfeld, 1996). Two prominent systems of orthogonally arranged, wide-field neurons are the horizontal-motion-sensitive (HS) and the vertical-motion-sensitive (VS) cells (Eckert and Bishop, 1978; for reviews, see Hausen, 1984, 1993). In these neurons, the directional sensitivity of local dendritic regions varies systematically across the retinotopic mosaic of the lobula plate, such that each of these neurons responds to rotatory motion about a particular axis (Krapp and Hengstenberg, 1996, 1997). Thus, each wide-field neuron may act as a "matched filter" for a specific type of visual stimulus.

In addition to retinotopic pathways from the medulla that supply wide-field lobula plate neurons, anatomical and physiological studies (Gilbert and Strausfeld, 1992; Strausfeld and Gilbert, 1992) have identified at least two more parallel pathways from the medulla that provide inputs to small-field retinotopic neurons in the lobula plate and in the lobula, the latter neuropil comprising palisades of small-field efferents ("Col" neurons) that are thought to be associated in part with chromatic processing (Strausfeld and Lee, 1991). These second and third parallel pathways are mediated by two very distinct populations of medulla outputs: 1) transmedullary (Tm) cells to the lobula alone, and 2) transmedullary cells with axons that

Grant sponsor: National Institutes of Health, National Center for Research Resources; Grant number: R01-RR08688.

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Received 29 August 1997; Revised 9 February 1998; Accepted 16 February 1998

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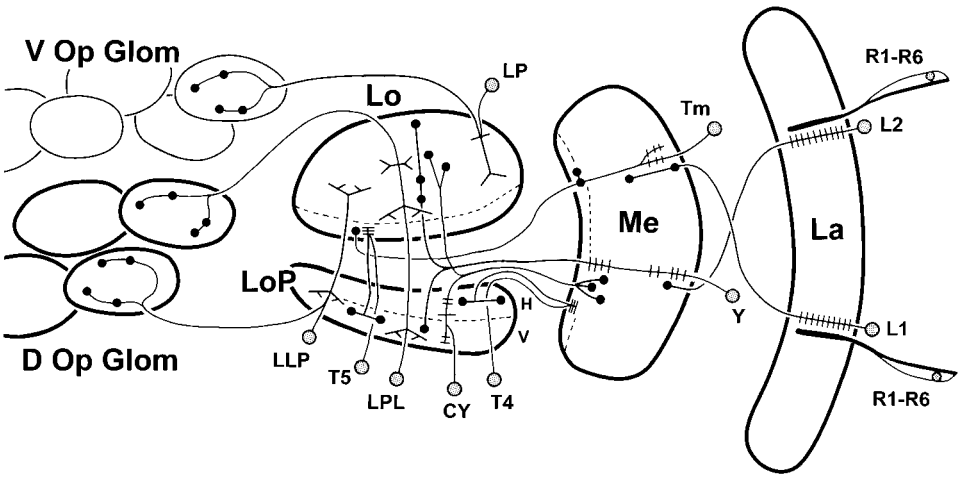


Fig. 1. Schematic summary of major cell types comprising retinotopic visual pathways in the calliphorid fly brain viewed as a horizontal section through the optic lobe (La, lamina; Me, medulla; LoP, lobula plate; Lo; lobula) and through several ipsilateral optic glomeruli (Op Glom) within the lateral deutocerebrum (after Strausfeld and Lee, 1991; Strausfeld and Gilbert, 1992; Douglass and Strausfeld, 1996). The anterior of the fly is toward the top of the page; dorsal (D) and ventral (V) refer to the neuraxis (see Materials and Methods, Terminology). Dendrites (thin bars) of large monopolar cells L1 and L2 receive achromatic inputs from photoreceptors R1–R6 and terminate (solid circles) at characteristic levels within the medulla, where a variety of retinotopic cell types project to the lobula and to horizontal (H) and/or vertical (V) motion-sensitive strata within the lobula plate. R7 and R8 photoreceptors (not shown) terminate at specific levels within the medulla (Bausenwein and Fischbach, 1992; Buschbeck and Strausfeld, 1996). Transmedullary (Tm) cells either 1) terminate at the outer layer of the lobula (as shown), also defined by dendrites of directionally selective T5 neurons, or 2) project to deeper lobula strata (see Fig. 14). Other columnar neurons intrinsic to the medulla (iTm; not shown) terminate at the innermost layer of the medulla, as defined by the dendrites of T4 neurons. Centripetal and centrifugal Y cells (Y and CY, respectively) make connections between the medulla, lobula, and lobula plate. Lobula plate-lobula neurons (LPL) and lobula-lobula plate neurons (LLP) terminate in the dorsal lateral deutocerebrum, whereas lobula projection neurons (LP) terminate ventrally.

bifurcate to provide terminals in the lobula and in the lobula plate. By virtue of their divided axons, neurons of this second pathway are called Y cells. They are organized retinotopically and are often characterized by elaborately decorated dendrites or terminals.

The branched axon of a Y cell relays information from a retinotopic area in the medulla to equivalent locations in both the lobula and the lobula plate (Strausfeld, 1970). Y cells are of particular interest, because their endings terminate at levels that coincide with the dendrites of a class of optic lobe output neurons that also have dendrites within both the lobula plate and the lobula. Depending on their orientation (axons leaving the lobula plate or axons leaving the lobula), these cells have been classified as lobula-lobula plate efferents (LLP neurons) or lobula plate-lobula efferents (LPL neurons; Strausfeld and Gilbert 1992).

Compared with wide-field lobula plate tangential cells, the functional roles of the LPL and LLP efferents are largely unknown. It is likely, however, that arrays of these small-field optic lobe outputs process particular stimulus categories, such as object position or tracking error, that must be represented in nervous system activity in order to generate the precise movements that characterize fly optokinetic behaviors. The idea that wide-field and small-field outputs must interact at follower neurons to provide for accurate adjustments of flight direction and head movements is suggested by their relationships with descending neurons, which supply neck and flight motor neuropils. LLP and LPL neurons invest the finer branches of these descending neurons, whereas wide-field, motion-sensitive outputs from the lobula plate contact their main trunks (Strausfeld and Bassemir, 1985).

This paper describes responses of neurons that participate in these small-field pathways. Activities range from simple responses to flicker, through more complex center-surround sensitivity, to responses to motion direction or motion speed. The present results demonstrate directional selectivity in cells that have an obvious dendritic bias in the lobula plate. Such neurons include a novel wide-field, amacrine-like lobula plate neuron and a small-field LPL neuron with terminals in the lateral deutocerebrum. Other, nondirectionally selective neurons in this pathway were intriguing in other ways. For example, tests using small-field stimuli suggest complex receptive field properties in LPL neurons and selectivity for motion speed or contrast frequency in LLP neurons. Responses of neurons that project from the medulla to the lobula or to both the lobula and the lobula plate (Tm and Y cells, respectively) can be divided into elements that are not motion- or direction-specific, suggesting that their functional roles involve other visual modalities such as motion speed, e-vector orientation, or chromatic properties, and other elements that show directional motion sensitivity.

The present results support the proposal (Gilbert and Strausfeld, 1992; Strausfeld and Gilbert, 1992) that small-field lobula plate neurons are involved in discrete aspects of visual processing that are important in motion perception, such as estimation of motion velocity, tracking of small object motions, and visual feature detection. Combined with observations of both direction- and nondirection-sensitive responses of Tm- and Y-cell inputs to the lobula plate, the results also support the concept of parallel pathways that filter distinct features of the visual environment (cf. Strausfeld and Lee, 1991; Bausenwein and Fischbach, 1992; Douglass and Strausfeld, 1996).

The Results section first describes small-field efferents that are shared by the lobula plate and the lobula. This is followed by a description of a new amacrine-like lobula plate neuron, and a discussion of morphological and physiological differences among neurons supplying both the lobula plate and the lobula (Y cells) and those supplying the lobula alone (Tm cells).

MATERIALS AND METHODS

Stimuli and electrophysiology

Methods for fly rearing, intracellular recording, and visual stimulus presentation have been detailed in previous publications (Douglass and Strausfeld, 1995, 1996) and are summarized here. Blow flies (*Phaenicia sericata*, Calliphoridae) are maintained as a laboratory colony at the Arizona Research Laboratories Division of Neurobiology. Eggs from locally trapped individuals are added to the colony several times per year to help maintain wild type levels of genetic diversity and fitness. Laboratory-reared *P. sericata* of either sex were immobilized with low-melting-point wax onto a pedestal fixed to a vibration isolation table (TMC, Peabody, MA). Single neurons were impaled within the medulla, the second optic chiasma, or the lobula complex by using sharp borosilicate pipettes back filled with Lucifer yellow and 0.1 M LiCl. During intracellular recordings, flies were stimulated with square-wave flicker and grating motion, which they viewed frontally either on a rear-projection screen (Douglass and Strausfeld, 1995) or on a computer-controlled RGB monitor (Vision Research Graphics, Durham, NH) viewed via a first surface mirror (see Douglass and Strausfeld, 1996). In experiments employing projected stimuli, the fly viewed a 108° circular, rear-projection screen that displayed either diffuse, wide-field flicker, or roughly constant-velocity motion in any of eight directions. The grating spatial frequency was of approximately 0.04 cycles per degree (cpd). Smaller field (40°) stimuli were produced by closing down an adjustable circular diaphragm (Melles Griot, Irvine, CA), which was placed at the rear focal plane of the grating image and could be precisely positioned within the stimulus field. The computerized display was 50° × 40° (width × height) and was operated at a horizontal refresh rate of 117 Hz with the three color guns set at equal luminance values. Circular flicker stimuli were presented at a frequency of 4 Hz, and full-screen grating motion (0.13 cpd or 8°/cycle) at sinusoidally varied velocities either from 0° to 93°/second or from 0° to 70°/second. In one experiment, a full-screen, constant-velocity bar motion stimulus (4° bar width, 37°/second) was used to rapidly assess the location of a cell's receptive field. Intracellular voltages and stimulus timing signals were amplified and stored on VHS tape with a VCR equipped with a PCM recording adapter (3000A; Vetter, Regensburg, PA). The raw data were also digitized on-line or during replay from the VCR, and were recorded to a PC (data-acquisition system; Datawave, Longmont, CO) for subsequent analysis.

Criteria for intracellular impalement and identification

A verbal record of events was recorded on the audio channel of the VCR during experiments. Penetration of a neuron was judged by a sudden voltage drop at the moment of penetration accompanied by the observation of unambiguous responses to flicker or motion or by the appearance of other characteristic changes in temporal voltage fluctuations (including, but not limited to, action potentials). As in other recordings from similar small, nonspiking peripheral neurons in dipteran optic lobes (Gilbert et al., 1991; Gilbert and Strausfeld, 1992; Douglass and Strausfeld, 1995), initial resting membrane potentials were typically smaller (circa -10 mV to –40 mV) than those observed commonly in recordings from larger neurons. Except where noted in the Results, a single recording was obtained from each identified cell, and all responses were confirmed by repeated presentations of the stimuli. Occasionally, small neurons that were penetrated briefly by the recording pipette were stained unintentionally with Lucifer yellow; thus, two neurons may have been stained in the same preparation if a second recording was obtained following an initial brief penetration. Typically, the two stained neurons could be assigned with confidence to their respective physiological recordings on the basis of their depths in the neuropil relative to the path taken by the recording pipette. In the few cases of double staining in which this assignment was uncertain, the physiological data have been omitted from the results.

Histology and anatomical reconstructions

At the end of each experiment, the recorded cell was injected iontophoretically with Lucifer yellow (1–2 nA steady hyperpolarizing current for 1–3 minutes; Molecular Probes, Eugene, OR), and the brain was removed from the head capsule and fixed in 4% formalin in Millonig's buffer, pH 7.2. Fixed brains were rinsed twice in Millonig's buffer, dehydrated in ethanol followed by acetone, and embedded in Spurr's (1969) plastic. In some preparations, after fixation and the initial buffer rinse, the cell bodies and tissue background were counterstained for 15 minutes with 2.5×10^{-5} M propidium iodide (Molecular Probes). Serial 14-µm sections were mounted on slides and coverslipped with Fluoromount (Serva, Heidelberg, Germany). Profiles of stained cells were scanned at 0.5-µm intervals with a confocal epifluorescence microscope equipped with an argon and a krypton/argon laser (MRC 600; Bio-Rad, Richmond, CA), or they were photographed at 1-µm intervals on a Leitz Diaplan epifluorescence microscope (Wetzlar, Germany).

Morphologies of filled neurons were reconstructed from epifluorescence photomicrographs and/or confocal projections of individual serial sections. Alternatively, confocal projections from separate plastic sections were merged by using image-processing software assisted by observations of stereo pairs (Confocal Assistant 3.1, Todd Brelje; Corel Photopaint 5 and 6; Corel, Salinas, CA) or three-dimensional reconstructions (NIH Image 1.57; Bethesda, MD). Anatomical relationships between intracellularly stained neurons and the surrounding neuropil architecture were revealed by using several methods, as illustrated in Figure 2A, in which a preparation containing a Lucifer yellow-stained Tm type 9 (Tm9) neuron was counterstained with propidium iodide. Separate Lucifer yellow (false-colored green) and propidium iodide (false-colored red) projections were obtained by using different lasers and filter blocks. Figure 2B was constructed as in Figure 2A, but was restricted to eight 0.5-µm optical sections, and the contrast and brightness values were increased in the propidium iodide image, thus demonstrating that the outer medulla dendrites of Tm9 occupy two adjacent retinotopic columns.

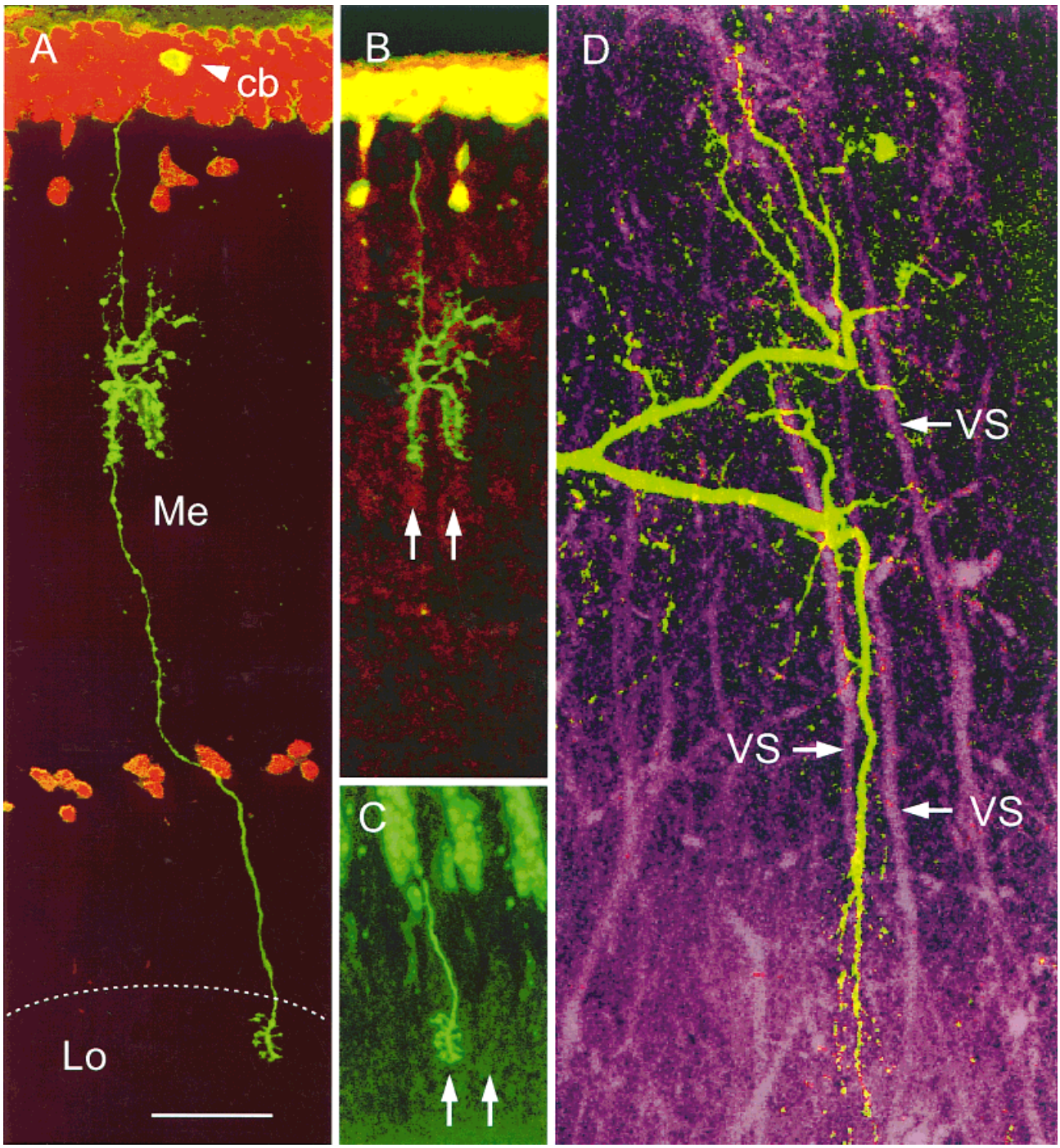


Fig. 2. **A–D:** Confocal reconstructions of transmedullary (Tm) neuron Tm9 (A–C) and a lobula plate-lobula (LPL) cell (D), illustrating methods employed to reveal relationships between intracellularly stained neurons and surrounding neuropil architecture (for details, see Materials and Methods). Both neurons were stained intracellularly with Lucifer yellow and reconstructed from confocal projections. The preparation containing Tm9 was counterstained with propidium iodide after fixation. Me, medulla; Lo, lobula; cb, cell body; VS, branches of three vertical motion-sensitive (VS) neurons in the lobula plate. Vertical arrows in B and C mark adjacent retinotopic columns. Scale bar = 20 μm in A (also applies to B,C), 18 μm in D.

In Figure 2C, high contrast and brightness values were applied to a single Lucifer yellow projection to reveal the relationship of the Tm9 terminal to the outer edge of the lobula (Fig. 2A, dashed curve). Figure 2D depicts the lobula plate arborizations of an LPL neuron, which is shown in its entirety in Figure 3E (see Results), in a preparation that was not counterstained. The LPL dendrites (false-colored green) were merged from successive confocal sections by using standard maximal projections, which preserve the maximal brightness value at each pixel

location. The same confocal series was then used to construct a minimal projection, preserving the darkest pixel values and thereby revealing the large nonautofluorescent shadows of VS cell branches (Fig. 2D, VS; false-colored purple).

Terminology

As in previous accounts (Strausfeld and Gronenberg, 1990), features within the central nervous system are described in relation to the longitudinal axis of the embryonic nervous system, which is turned upward in the adult brain. Thus, dorsal structures in the adult central nervous system, such as the lobula plate and the dorsolateral deutocerebrum, face caudally with respect to the ventral nerve cord.

RESULTS

General organization of small-field efferents from the lobula plate

Anatomical and physiological studies of the dipteran optic lobes (Strausfeld and Lee, 1991) have demonstrated the segregation of pathways from the retina into two main streams, both of which project through the medulla and then segregate out to the two deepest retinotopic regions of the optic lobes, the lobula plate and the lobula (Fig. 1). The lobula plate is a tectum-like neuropil that is characterized by layers of wide-field, directional motion-sensitive neurons. The lobula is a deep, lima bean-shaped center that is characterized by ensembles of columnar neurons. Anatomical studies indicate that the lobula receives relays from the medulla that are associated with putative color-processing pathways, whereas the lobula plate receives achromatic inputs (Strausfeld and Lee, 1991; Bausenwein and Fischbach, 1992).

The lobula plate is supplied by small retinotopic afferents arising from the medulla. These are the bushy T cells (termed T4 and T5 cells), each of which sums information from a group of elementary motion-detector circuits that comprise a system of relays from the retina and lamina, intermediate Tm neurons, and centrifugal and amacrine cell pathways (Douglass and Strausfeld, 1995). The bushy T cells terminate on HS and VS neurons (and other tangentials) that collate information about motion and that play a crucial role in visually stabilized flight (Hausen, 1984, 1993; Gilbert et al., 1995).

In addition to tangential neurons, small-field neurons from the lobula plate, which also have branches in the lobula, project to glomerulus-like regions ("optic foci" or "optic glomeruli"; Strausfeld, 1976; Strausfeld and Gronenberg, 1990) within the lateral deutocerebrum (Fig. 1) into which descending neurons that supply neck and flight motor neuropils send dendritic branches. The axonal trunks of these descending neurons receive inputs from wide-field, motion-sensitive lobula plate tangentials. Thus, descending pathways that play a crucial role in optokinetic behaviors (Gilbert et al., 1995; Gronenberg et al., 1995) receive information from two converging systems: wide-field, panoramic motion-sensitive elements and small, retinotopic efferents (Strausfeld and Bassemir, 1985; Strausfeld and Gilbert, 1992).

Efferents shared by the lobula plate and the lobula supplying the lateral deutocerebrum (LPL and LLP cells)

Directionally selective LPL neurons. In two separate preparations (Fig. 3A,E), a recording was obtained from a wide-field LPL cell type that has been identified previously in another muscoid fly (*Sarcophaga bullata*) and termed "LPL-67" (Gilbert and Strausfeld, 1992). The basic morphology of this neuron is remarkably consistent. As Gilbert and Strausfeld (1992) have also noted, the trunk of this LPL neuron bifurcates vertically on its way from the lobula to the lobula plate, and the two subsequent branches provide a narrow, vertical dendritic field within the VS layer of the lobula plate (see Fig. 1). A confocal reconstruction of the neuron, adjusted to enhance the nonfluorescent shadows of VS cell branches (Fig. 2D), shows that the dendrites of this LPL neuron are closely associated with the posterior (lower) branches of three VS cells that were identified on the basis of their positions in the lobula plate as either VS3–VS5 or VS4–VS6. The vertically oriented lobula plate tributaries of the LPL neuron, as estimated by the bundles of input fibers arriving at the lobula plate from the medulla, span approximately ten retinotopic columns. The lobula arborizations of this LPL neuron invade fewer retinotopic columns than the branches in the lobula plate. Thus, the overall organization is suggestive of a center dendritic field provided by the lobula branches and a surround organization provided by the oval, vertically oriented dendritic field in the lobula plate.

The terminal arborizations of this neuron lie within an egg-shaped volume of neuropil that is situated just lateral and slightly dorsal to the esophagus in the ipsilateral deutocerebrum. In a Golgi study of muscoid flies, LPL cells have been categorized morphologically into eight basic types (Strausfeld and Gilbert, 1992). By virtue of the bifurcated axon and unistratified projection into the lobula plate, the two neurons illustrated in Figure 3 are classified as type 7 LPL cells.

In our first recording from this type of LPL neuron (Fig. 3A), background activity consisted of small, transient depolarizations. Flicker responses (Fig. 3B) were ON-hyperpolarizations and OFF-depolarizations, similar to those observed in many dipteran visual interneurons (cf. Laughlin, 1981; Douglass and Strausfeld, 1995, 1996). Both vertical and horizontal grating motions (Fig. 3C,D) hyperpolarized the membrane potential and inhibited the background transient depolarizations, and no consistent evidence for directional selectivity was observed. Responses were quite different in the second preparation (Fig. 3E), in which the frequency of spike-like depolarizations increased during motion. Although the intracellular voltage record was lost, replays of the audible spiking activity on the VCR voice channel provided a qualitative record of the responses to motion. The frequency of depolarizing activity was higher during progressive motion than during regressive motion, clearly demonstrating selectivity to the direction of motion. Gilbert and Strausfeld (1992) also reported directionally selective responses from "LPL-67." Thus, despite its nondirectional activity under some recording conditions (Fig. 3C,D), clearly, this cell type is capable of directionally selective responses.

Type 9 small-field LPL neuron. This small-field LPL neuron (Fig. 4) has dense arborizations in the lobula plate

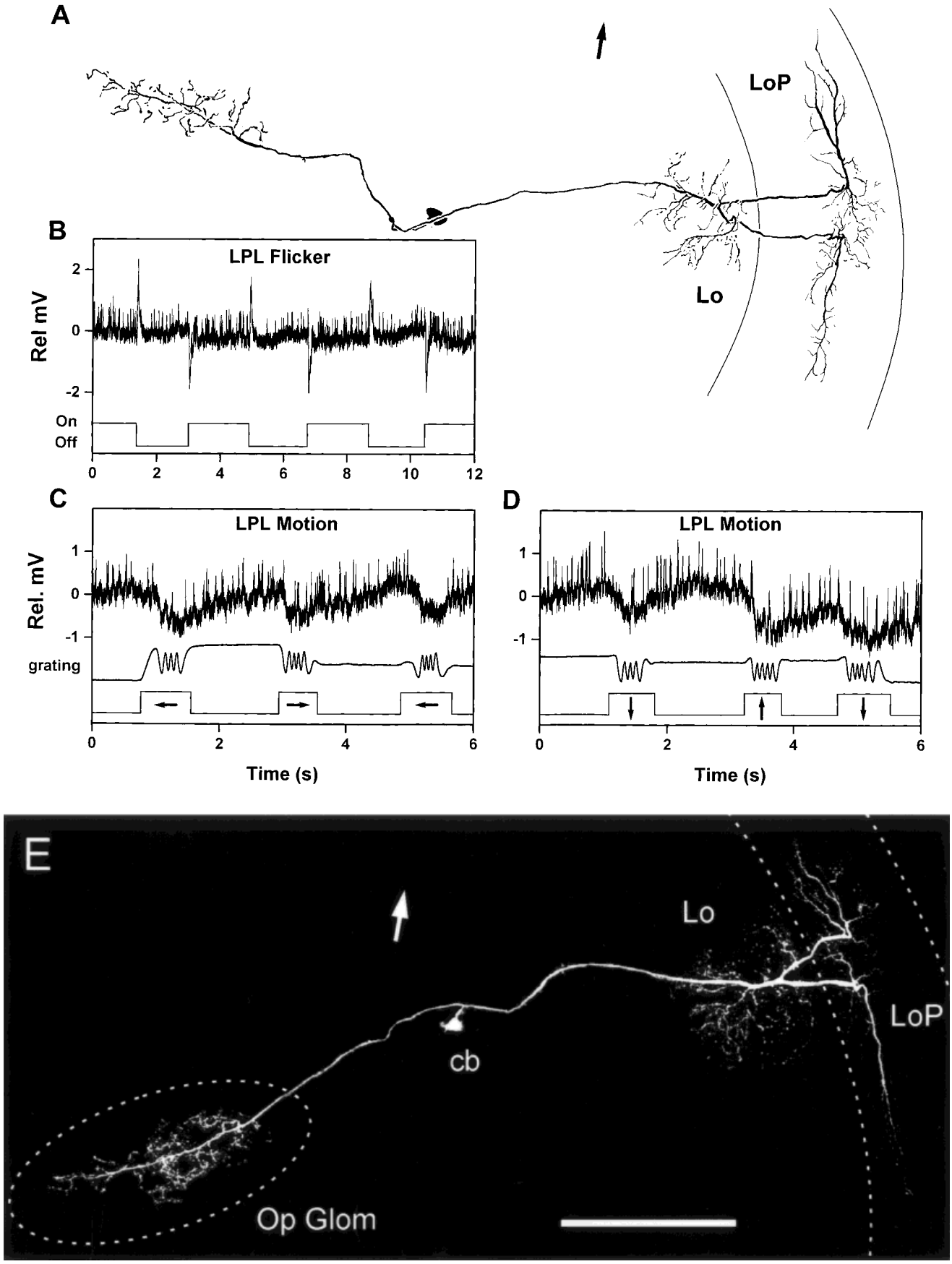


Fig. 3. Two separate preparations in which intracellular staining and recordings were obtained from the same identified lobula plate-lobula (LPL) type. LoP, lobula plate; Lo, lobula, Op Glom, optic glomerulus; cb, cell body. **A:** Anatomical reconstruction from frontal sections of the first preparation. **B–D:** Responses of this cell to projected wide-field flicker (B) and grating motion (C,D). **E:** The second LPL of this type, which was reconstructed from confocal projections of frontal sections. Other than having different anatomical receptive fields within the lobula plate (LoP), the basic features of the two stained LPL cells are virtually identical. Arrows (A and E) indicate the anterior direction (dorsal with respect to the head). Scale bar = 80 µm in E, 85 µm in A.

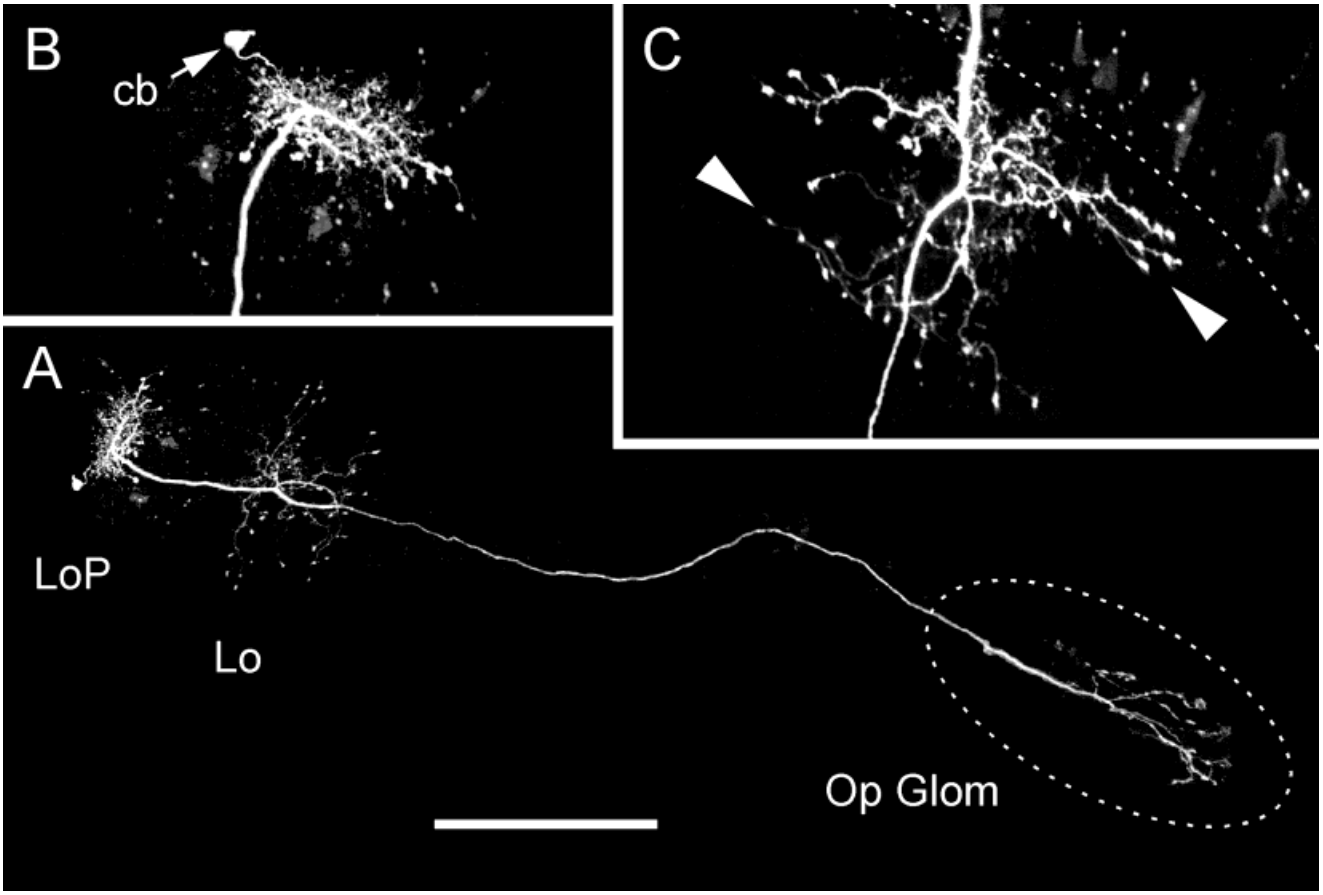


Fig. 4. **A-C:** Reconstructions from 15 confocal projections of a lobula plate-lobula (LPL) neuron (A) characterized by a small field in the lobula plate (B), bistratified lobula arborizations (C, arrowheads) and terminals in an optic glomerulus (A; Op Glom) near the posterior lateral edge of the dorsal deutocerebrum. Dashed lines indicate the approximate outlines of an optic glomerulus in A and the outer edge of the lobula in C. LoP, lobula plate; Lo, lobula; cb, cell body. Scale bar = 40 µm in A, 20 µm in B,C.

that span approximately five columns vertically and two columns horizontally, with more diffuse branching in the lobula. The neuron terminates in an ipsilateral optic glomerulus of the posterior dorsolateral deutocerebrum. The lobula plate arbors exhibit predominately spiny dendrites with occasional varicose profiles. The lobula morphology is complex. Mainly spiny arbors are clustered close to the axon, and there are two distinct strata of varicose arborizations that extend tangentially to each side of the axon. Because the bistratified morphology in the lobula is unlike any of the eight LPL neuron types described previously (Strausfeld and Gilbert, 1992), we identify this as a "type 9" LPL neuron. The deutocerebral terminal appears exclusively varicose, suggesting that the cell derives its input in the lobula plate, has complex synaptic relationships in the lobula, and has terminals in the central brain that are presynaptic.

The flicker responses of this LPL cell (Fig. 5A) were typical ON-hyperpolarizations with a sustained hyperpolarizing plateau and OFF-depolarizations during each flicker cycle. With 10-Hz flicker (Fig. 5A, left), the overall amplitude of intracellular voltage fluctuations was reduced only slightly compared with 2-Hz flicker. Wide-field grating motion (Fig. 5B,C) produced weak fluctuations at the grating contrast frequency superimposed upon sustained hyperpolarizations that recovered rather slowly. No evidence for direction or orientation selectivity was observed. The hyperpolarizing motion responses, however, differed dramatically from the voltage fluctuations produced during flicker at similar (approximately 4 Hz) contrast frequencies, suggesting that this LPL neuron can discriminate wide-field flicker from motion, or at least from complex spatiotemporal patterns of intensity changes.

Type 1 LPL neuron. Figure 6 illustrates a small-field LPL cell with lobula arborizations that traverse through half of the depth of this neuropil. The neuron provides terminals in an optic glomerulus within the lateral deutocerebrum. Based on its small lobula plate field and nonstratified arbors that span only the outer half of the lobula, this neuron most closely resembles the LPL type 1 neuron (Strausfeld and Gilbert, 1992). The neuron was stimulated initially with wide-field (108°) flicker and motion, followed by tests using a smaller stimulus field (40°). Stimulation with wide-field flicker (Fig. 6A) elicited typical large, monopolar cell (LMC)-like ON-hyperpolarizations and OFF-depolarizations, with additional, small voltage fluctuations during light ON. Wide-field grating motion (Fig. 6B) produced small, mainly depolarizing voltage fluctuations at the grating contrast frequency; these responses were also very similar to those of LMCs (Gilbert et al.,

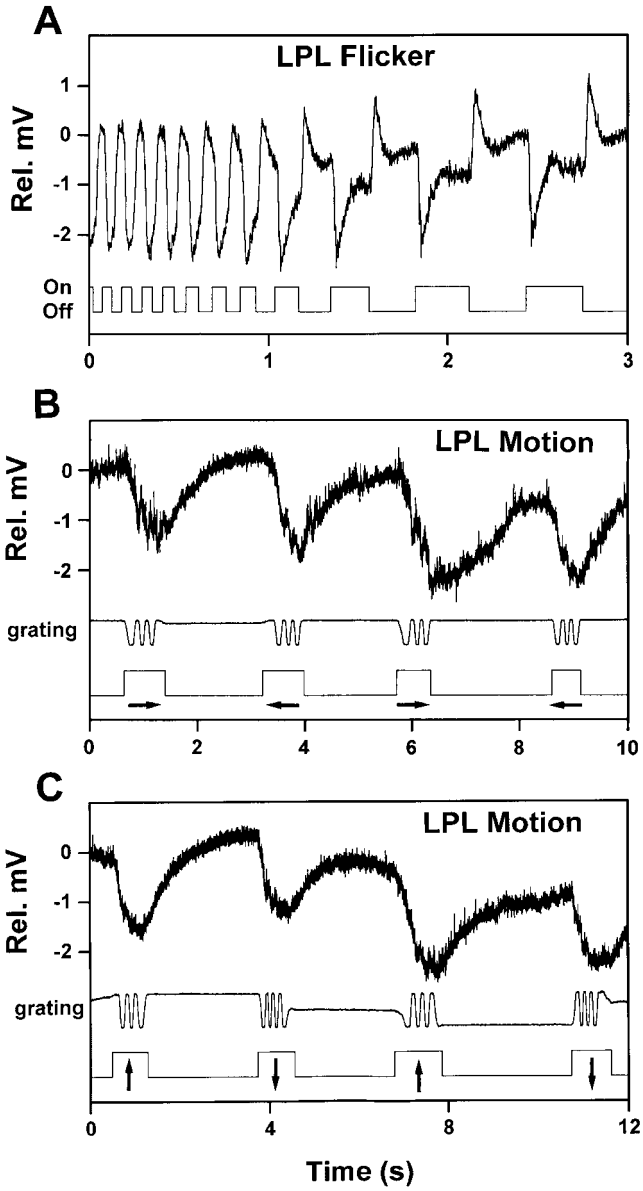


Fig. 5. Distinct responses of the lobula plate-lobula (LPL) neuron shown in Figure 4 to flicker (**A**) and to directional grating motion (**B,C**).

1991). Tests at eight different motion directions (data not shown) showed no evidence for directional selectivity. Responses to the smaller field motion, however, were dependent on stimulus location in a nonlinear fashion. In a mediodorsal and an ipsilateral dorsal field (Fig. 6C; also locations indicated by minus signs in the inset above Fig. 6D), small-field vertical motion produced sustained hyperpolarizing responses, whereas motion in a ventral field (Fig. 6D and plus signs in inset) produced transient depolarizations that were indistinguishable from the wide-field responses. Motion in the contralateral visual field had no effect ("0" in inset above Fig. 6D). The same pattern of excitatory and inhibitory responses was observed during repeated presentations of the small-field vertical motion stimulus and persisted during small-field motion in other directions.

Type 4 LLP neuron exhibiting contrast-frequency dependence. LLP neurons have their distalmost arborizations in the lobula and project to the lateral deutocerebrum via axons that leave the inner surface of the lobula plate. Two similar LLP neurons were stained in a preparation in which only one neuron was filled intentionally (Fig. 7). Because their unistratified lobula and lobula plate arborizations are displaced from one another in the retinotopic mosaic, these are identified as type 4 LLP neurons (Strausfeld and Gilbert, 1992). Although their close proximity and similar staining intensities preclude an unequivocal assignment of the intracellular recording, visual inspection showed that the neuron on the left was more brightly filled, and thus should correspond to the recorded cell. Examination of stereo pairs, which were constructed from confocal projections of the terminal regions of these two neurons, showed that they target different regions of the same optic glomerulus (Fig. 7C). The cell on the left arborizes deep within the core, whereas terminals of the second LLP (Fig. 7C, arrows) wrap around the outer edges of the glomerulus.

Flicker responses (Fig. 7A) were ON-OFF depolarizations, unlike the LMC-like flicker responses noted above from LPL neurons. The intracellular voltage was somewhat unstable during stimulation with grating motion at sinusoidally varied speeds. Tests at all eight motion directions failed to reveal any clear pattern of directional selectivity, but transient depolarizations were clearly associated with changes in contrast frequency, suggesting that this neuron may also be selective for motion speed. The transient depolarizations were observed most consistently during slow motion (Fig. 7B) at angular velocities less than 50°/second (equivalent to contrast frequencies less than 6.5 Hz), and were absent both during the fastest motion (50–93°/second) and whenever motion had ceased.

Local tangential neurons

The dipteran lobula plate is well known for its variety of giant, directional motion-sensitive, tangential lobula plate afferents that, corresponding to phylogenetic position and/or body morphology and flight behavior, have receptive fields ranging from large to panoramic (Buschbeck and Strausfeld, 1997). Many of these neurons in the optomotor pathway (e.g., HS and VS cells) have terminals in the ipsilateral dorsal deutocerebrum, where they are closely intertwined with, and often show cobalt coupling to, large descending neurons that lead to neck and flight motor neuropil in the dorsal thoracic ganglia (Strausfeld and Bassemir, 1985; Strausfeld and Lee, 1991). Other giant lobula plate tangential neurons terminate in the contralateral lobula plate (H1, V2, and V3) and the contralateral deutocerebrum (H2–H4, V1, and CH: Hausen, 1984; H6: Douglass and Strausfeld, 1996; "Hx": Krapp and Hengstenberg, 1996), where they make inhibitory connections with premotor descending neurons (Gilbert et al., 1995; Gronenberg et al., 1995; Strausfeld et al., 1995).

Despite the variety of identified wide-field lobula plate neurons, only rarely have elements been stained that are intrinsic to the lobula complex, and, until now, none has been interpreted functionally (see Discussion). We have

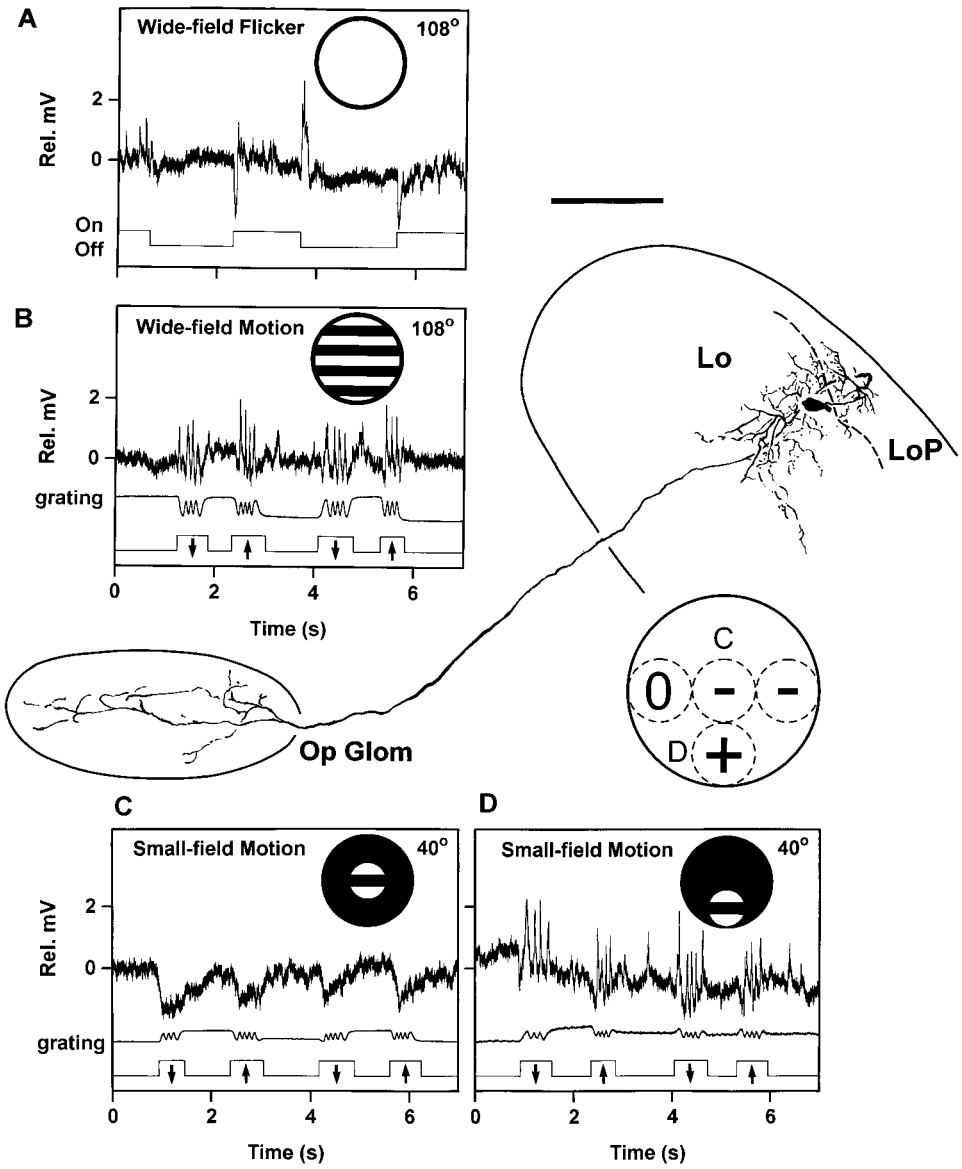


Fig. 6. Type 1 lobula plate-lobula (LPL) neuron with complex receptive field properties. **Insets** above each recording indicate the size and position of each stimulus. Responses to wide-field (108°) flicker (**A**) and motion (**B**) showed no evidence for directional selectivity. Responses to smaller field (40°) motion were dependent upon stimulus location. In dorsal (**C**) and ipsilateral flanking fields (minus signs in inset above D), small-field motion produced hyperpolarizing responses, whereas motion in a ventral field (**D** and plus sign in inset above D) produced depolarizations. Contralateral motion produced no response (0 in inset). Scale bar = 30 µm.

recorded from and stained one such neuron (Fig. 8), which we refer to as an "intrinsic LPL." This neuron (Fig. 8D) has a very extensive bistratified field in the lobula plate and a relatively small-field projection to the ipsilateral lobula. The largest arborizations of this cell consist of two vertical branches, both of which straddle the middle strata of the lobula plate, where dendritic inputs can be expected from both the vertically sensitive and the horizontally sensitive layers of this neuropil. Also within the lobula plate, a small, secondary arborization projects anteriorly (upward) into the outer, horizontally sensitive layer of the plate (Fig. 8D, open arrow). The lobula projection is somewhat larger than the lobula plate's secondary arbor, but otherwise represents a similar retinotopic region.

The flicker responses of this neuron (Fig. 8A) were ON-hyperpolarizations that recovered within approximately 100 msec and OFF-depolarizations that recovered somewhat more gradually. Instead of the hyperpolarizing plateau that is often observed in intracellular recordings from the optic lobe during light ON, the baseline recovered completely and was followed by sustained, small, transient depolarizations that continued for at least 1 second. Motion responses (Fig. 8B,C) were characterized by direction-dependent, sustained hyperpolarizations and depolarizations. The largest depolarizations occurred during regressive and downward motion, as illustrated in a polar plot of the differences between mean intracellular voltages before and during motion (Fig. 8E).

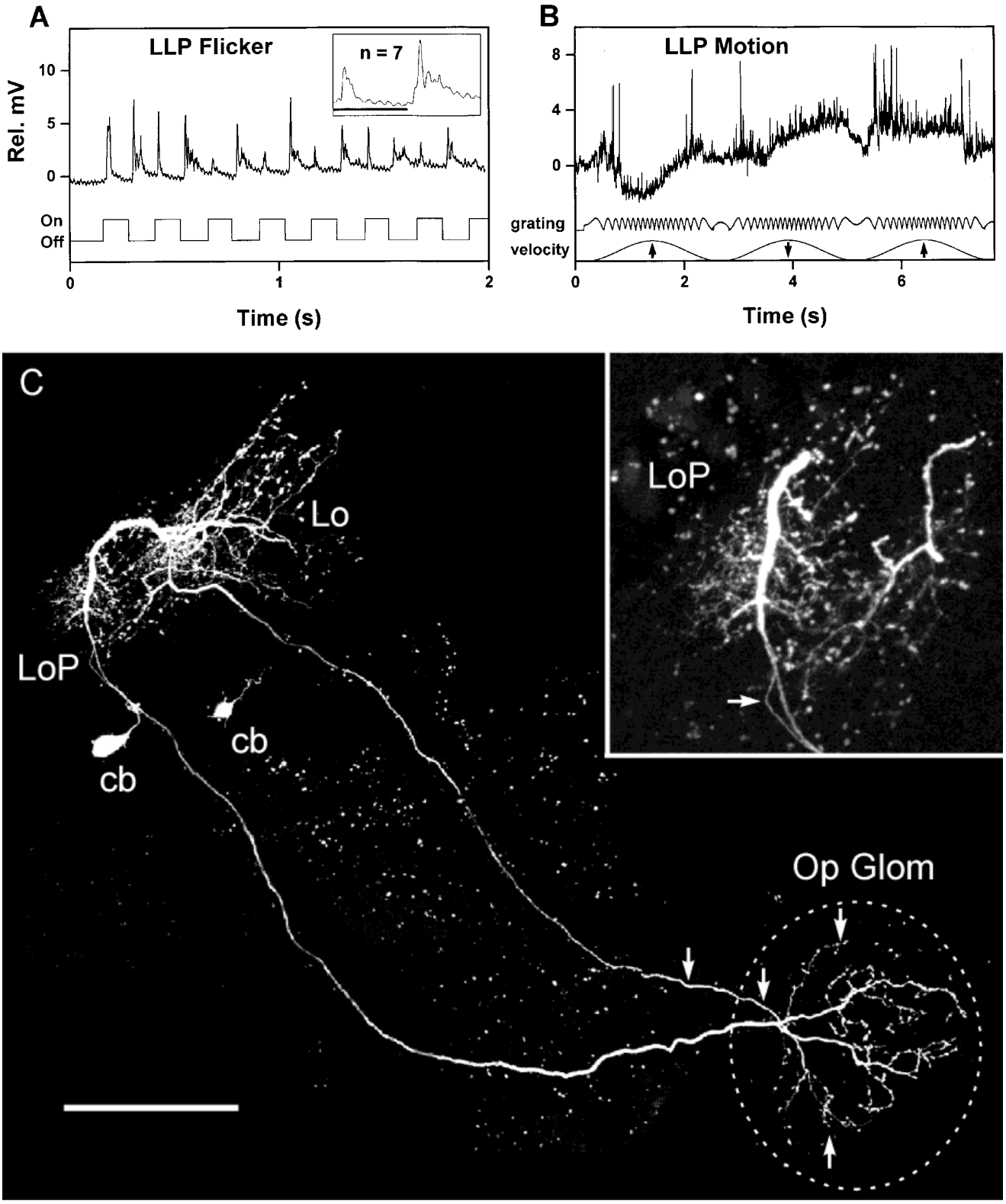


Fig. 7. Two lobula-lobula plate (LLP) efferent neurons that target different regions of the same optic glomerulus (Op Glom). **A:** Responses to 4-Hz flicker. **Inset** shows mean response (bar = light ON) and demonstrates a slightly larger amplitude depolarization to OFF than to ON. **B:** Responses to vertical wide-field motion with sinusoidally varied velocity from 0° to 93°/second illustrate a preference for motion at low-contrast frequencies. **C:** Reconstruction of both stained neurons from 18 confocal projections. Terminals of the more faintly stained cell (arrows) surround those of the more brightly filled cell. **Inset:** Detail of lobula plate showing cell body fiber (arrow) of the left neuron. Scale bar = 40 μm in C, 20 μm in C inset.

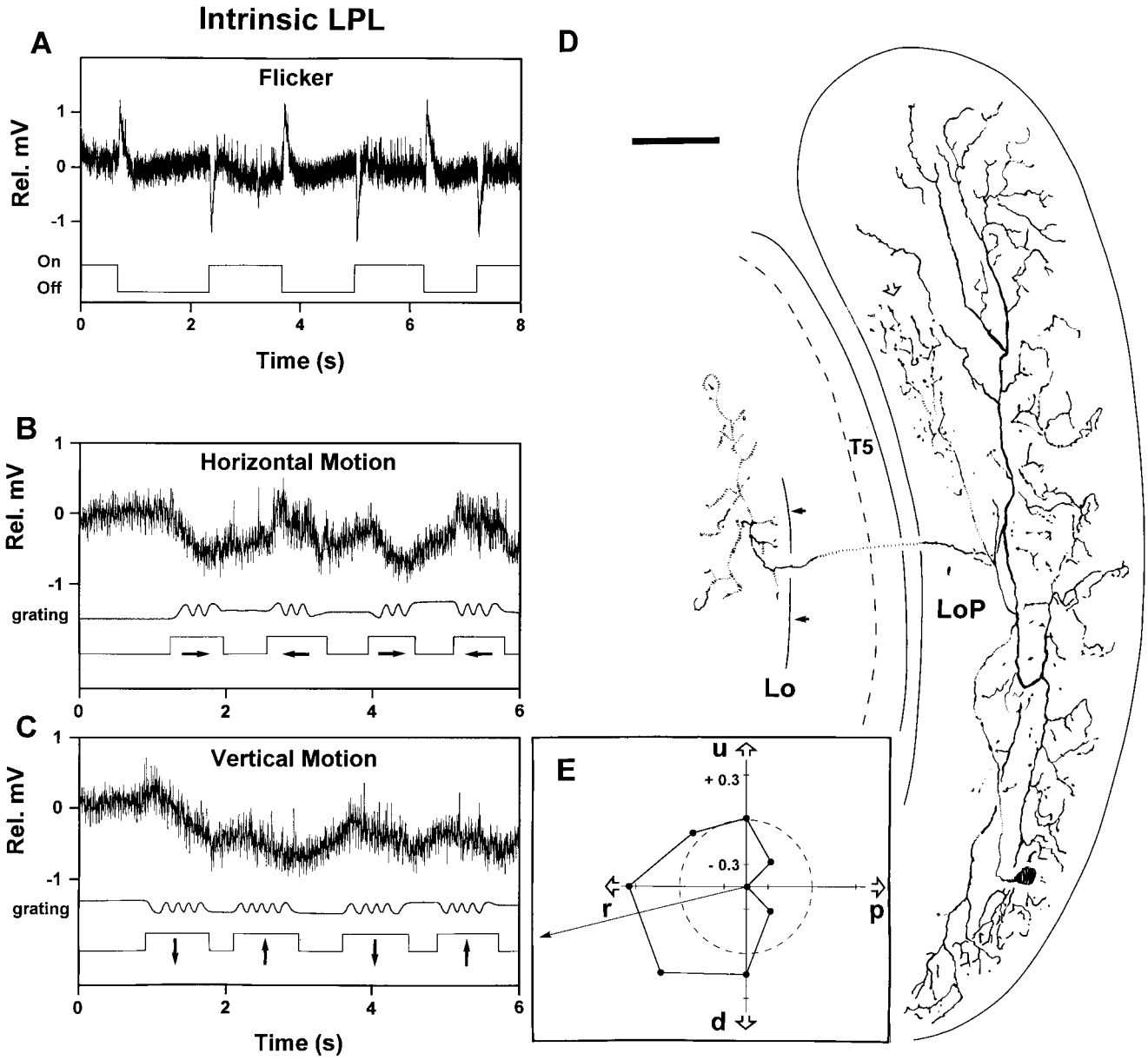


Fig. 8. **A–E:** A directionally selective neuron (D) that is intrinsic to the lobula complex, with two distinct strata in the lobula plate (LoP): a large, wide-field arborization and a small-field arborization (open arrow at upper left of the lobula plate profile) in the horizontal motion-sensitive stratum of the lobula plate (see Fig. 1). A smaller zone of lobula arbors is at the outer limit of Col A cell dendrites (solid arrows). A: Flicker responses. B,C: Representative responses to directional motion. E: Differences in mean intracellular voltage (mV) 500 msec after and 50 msec before the start of motion in each direction. Dashed circle shows the zero-response level, representing no net change in intracellular voltage. The cumulative response vector (long solid arrow) shows strongest depolarizations to regressive, slightly downward motion. p, Progressive; r, regressive; u, upward; d, downward. Scale bar = 50 µm in D.

Medulla connections to the lobula and lobula plate: Y cells

Y cells (see Fig. 1) are a heterogeneous class of tripartite neurons that arborize in the medulla, lobula, and lobula plate. The Y cells, as a group, are thought to represent as much as 80% of the inputs to the lobula plate from the medulla (Strausfeld, 1976), yet their physiological properties are virtually unknown. Very few recordings to date have been identified unambiguously with Y cells, possibly because their thin axons are difficult to penetrate with intracellular pipettes. Most members of this class are considered to be centripetal neurons, because their perikarya are located distal to the medulla, and light microscopy shows that their arborizations within the lobula complex are well endowed with varicose and beaded profiles that are indicative of presynaptic swellings (see Strausfeld and Bassemir, 1985). A handful of morphologically centrifugal Y cells has also been identified (Strausfeld, 1976; Fischbach and Dittrich, 1989; Douglass and Strausfeld, 1996); in each of these, the cell body is located

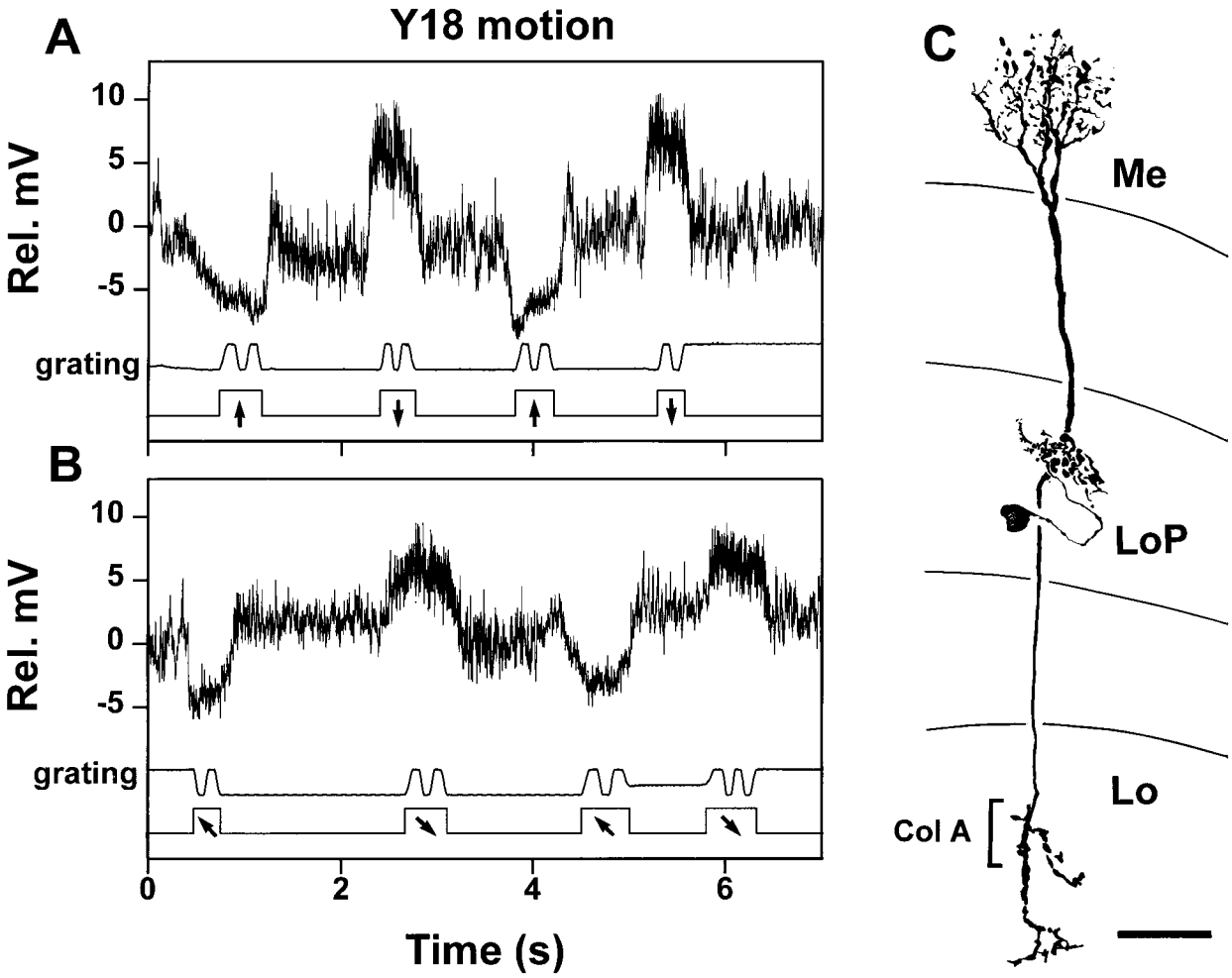


Fig. 9. Motion responses in a centrifugal Type 18 Y cell (Y18). Directional selectivity is demonstrated by the sustained depolarizations during downward (**A**) and downward-progressive (**B**) motion, in contrast with hyperpolarizing responses to upward and upward-progressive motion. **C:** Anatomical reconstruction from fluorescence photomicrographs. Me, medulla; LoP, lobula plate; Lo, lobula; Col A, lobula stratum occupied by dendrites of Col A cells. Scale bar = 20 μm.

behind the lobula plate, and the medulla arborizations are restricted to beneath the serpentine layer. There is a variety of centripetal, small-field transmedullary Y cells, each of which appears to be represented in every retinotopic column. Other centripetal Y cells have wider field arborizations and may occur only once every few columns.

Type 18 Y cell (Y18). The centrifugal Y cell shown in Figure 9 corresponds closely to Golgi observations of *Musca* Y18 neurons (Strausfeld, 1976) or to the Y3 cells in *Drosophila* (Fischbach and Dittrich, 1989). A single recording from *Sarcophaga* (Gilbert et al., 1991) illustrates a cell that is superficially similar to the present Y18 cell (and was classified as Y18 by Gilbert et al., 1991) but has significant morphological differences from the cell described below (see Discussion).

The most distinctive feature of the present type 18 Y element is its roughly symmetrical arrangement of short branches extending into the inner layers of the medulla from its inner surface. Some five to six main branches provide processes that spread through a symmetrical domain, spanning a diameter of approximately seven retinotopic columns. The processes are suggestive of both input and output synapses: They do not obviously taper and are invested with both spines and large varicosities. Notably, finer processes predominate proximally, at a level corresponding to medulla layer 9 (M9; Fischbach and Dittrich, 1989), and most of the varicosities are located distally, at layer M8. The lobula plate arborizations are located within the deeper parts of the neuropil, that is, at levels corresponding to vertical motion sensitivity. As in the medulla, both types of processes occur, but here, they do not appear to be spatially segregated. In the lobula, the terminal of Y18 is bistratified, the outer stratum corresponding to the level of Col A dendrites (Strausfeld and Hausen, 1977). Both strata are sparsely branched, showing little evidence of dendritic spine-like specializations.

Observations of the tract made by the penetrating electrode suggested that the recording from Y18 was from the axon connecting the medulla to the neuron's lobula plate component. The neuron responded to motion in a directionally selective manner, showing nonspiking, sustained hyperpolarizations to upward motion and depolarizations to downward motion (Fig. 9A). Similar hyperpolar-

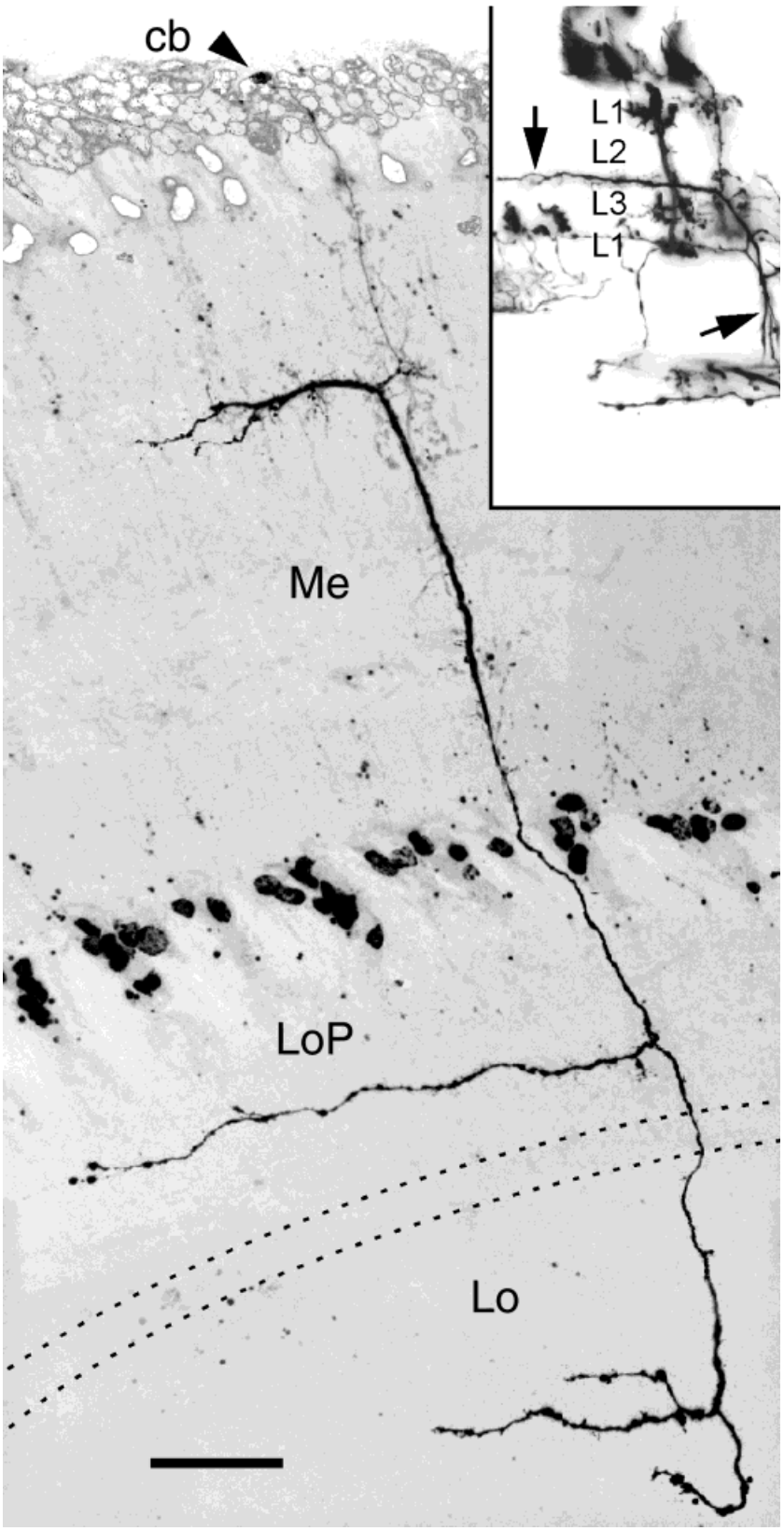


Fig. 10. The centripetal type 17 Y cell (Y17) reconstructed from merged confocal projections. Dashed lines indicate the chiasma between the lobula plate and the lobula. Y17 was stained intracellularly with Lucifer yellow, and the neuropil background and other cell bodies were counterstained with propidium iodide. The gray-scale image has been inverted. Thus, stained structures appear dark except for propidium iodide-stained somata distal to the medulla (pale globular profiles), which were left uninverted to contrast with the Lucifer yellow-stained Y17 cell body (cb). **Inset:** Golgi preparation illustrating the relationship between the outer medulla arbor of Y17 (arrows) and the medullary strata defined by large-lamina monopolar cells L1, L2, and L3. Me, medulla; LoP, lobula plate; Lo, lobula. Scale bar = 20 μm.

izing responses were obtained with upward-progressive (45° from horizontal) motion, and depolarizations during downward-regressive motion (Fig. 9B).

Type 17 Y cell (Y17). Figure 10 illustrates a distinctive centripetal Y cell known from both *Eristalis* (Strausfeld, 1976) and *Drosophila* (Fischbach and Dittrich, 1989).

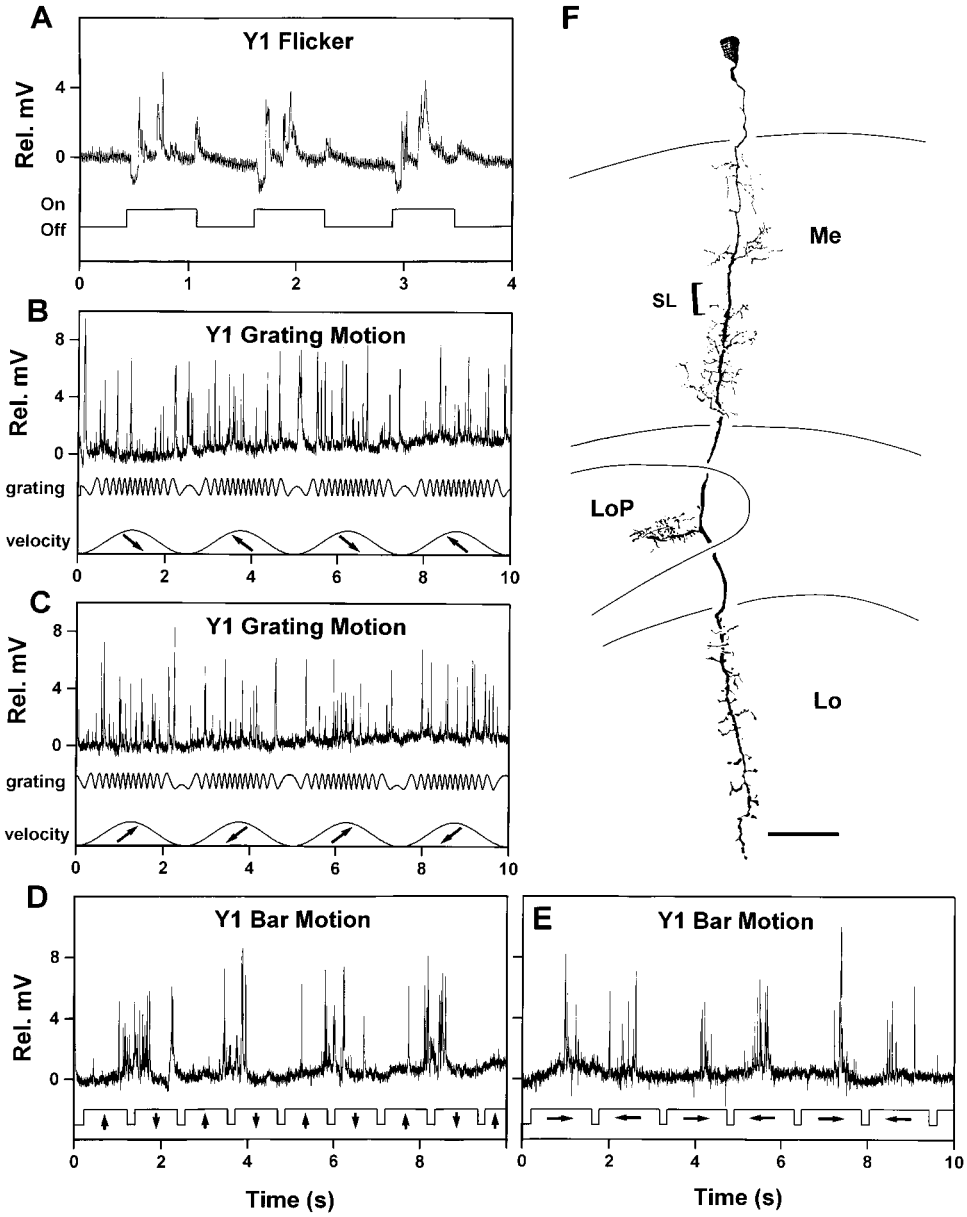


Fig. 11. Y1, a small-field transmedullary centripetal Y cell. **A:** Flicker responses. **B,C:** Responses to diagonal wide-field grating motion with sinusoidally varied velocity from 0° to 70°/second. **D,E:** Responses to constant-velocity, vertical (D) and horizontal (E) motion of a single white bar against a dark background. The stimulus began as a stationary horizontal bar at the bottom of the fly's view of the screen (D) or a vertical bar at the left side (E), and was moved across the screen at 37°/second with 170 msec pauses between sweeps. **F:** Anatomical reconstruction from fluorescence photomicrographs showing Y1 morphology in the medulla (Me), lobula plate (LoP), and lobula (Lo). SL, serpentine layer of the medulla. Scale bar = 25 μm.

Unlike many centripetal Y cells, which have a columnar retinotopic organization in each neuropil of the lobula complex and are bi- or multistratified in the medulla, Y17 is organized tangentially, and its only major medulla arborization is restricted to the L3 large monopolar cell layer at the level of line amacrines (Strausfeld, 1970). This preparation yielded excellent staining but no reliable responses to either motion or flicker. The medulla arbors extend vertically across some seven retinotopic columns, a single elongated tangential branch transects much of the lobula plate, and the lobula projection gives rise to three terminals deep within this neuropil. It is noteworthy that each tangential trunk has a similar directional orientation within the retinotopic map of its respective neuropil. In the present example, this orientation is vertical, with the ends of the branches extending in the direction that corresponds to downward in the visual field. The lobula plate projection lies within the inner, VS cell layers, as demonstrated in confocal reconstructions (not shown) in which the shadows of the VS cells were enhanced (cf. Fig. 2D).

Type 1 Y cell (Y1). Figure 11 depicts a small-field, transmedullary centripetal Y cell with spiny arborizations at several levels in the medulla. In the lobula, this neuron provides small-field, blebbed arborizations that extend through an entire retinotopic column, and its branch into the lobula plate extends to a point slightly deeper than the

HS layer. The medulla arborizations are well developed in the deep L1 layer of the outer medulla and throughout most of the inner medulla, but they are excluded from the serpentine layer. The overall morphology of this neuron closely matches that of Y1 from *Musca* (Strausfeld, 1976), and we identify it as such, although the lobula plate projection in *Musca* appears to transect both the HS layer and the VS layer.

Y1 was stimulated with computer-generated motion and flicker. Its flicker responses (Fig. 11A) consisted of an initial ON-hyperpolarization followed by depolarizations during illumination and upon light OFF. During sinusoidally modulated grating motion, small, variable-amplitude, transient depolarizations occurred (Fig. 11B,C). These were similar to the depolarizing activity during flicker ON, but without the ON-hyperpolarizations that also characterized flicker responses at a similar contrast frequency. The motion-induced transients showed no consistent pattern in relation to either the direction or the contrast frequency of grating motion. Movement of a single bright bar (4° wide) against a dark background also elicited transient depolarizations, with no large or consistently timed hyperpolarizations (Fig. 11D,E). Thus, Y1 exhibited no directional selectivity but did show subtle differences in its responses to motion and flicker. In addition, the responses to bar motion suggest an excitatory receptive field within the upper half of the right frontal visual field. The depolarizations during bar movement usually occurred as the bar traversed a right frontal region (Fig. 11E) near the upper edge of the stimulus field (Fig. 11D). This functional identification of the receptive field is consistent with its predicted location based on the retinotopic positions of its arborizations within the right optic lobe.

Medulla afferents to the lobula: Tm cells

Small-field Tm cells (Figs. 1, 2A–C, 12–14) are retinotopic elements that traverse the entire depth of the medulla and terminate at various levels within the lobula (Cajal and Sanchez, 1915). Tm1 (Calliphoridae; Strausfeld, 1976) and Tm9 as well as a variant termed Tm1a (*Drosophila*; Fischbach and Dittrich, 1989) are characterized by extensive arborizations at the L2/L3 layers of the outer medulla and an absence of arborizations in the L1 layers. These are also the only consistently observed lobula afferents in Diptera that have terminals restricted to the T5 dendritic layer in the outermost stratum of the lobula (Fig. 2C). The only difference noted to date between Tm1 and Tm9 is that Tm1 has well-developed axon collaterals in the proximal medulla, just distal to the T4 dendritic layer, whereas Tm9 has no arborizations in the proximal medulla at all. Because Tm1 and Tm9 share the same basic morphology, and Tm9 has been observed only rarely in Golgi preparations of *Drosophila*, Fischbach and Dittrich (1989) acknowledged that the latter cell type might merely represent a morphological variant of the former. On the other hand, because the Golgi method often underrepresents certain cells (Strausfeld, 1980), Tm9 may only appear to be rare.

Type 9 Tm cell (Tm9). A recording from Tm9 in *Phaenicia* (for Tm9 reconstruction, see Fig. 2A–C) now confirms that this is a unique Tm type that is distinct both morphologically and physiologically from Tm1. In their extensive comparative study, Buschbeck and Strausfeld (1996) noted that the lobula terminals of Tm1 are branched only sparsely. In contrast, the terminals of Tm9 exhibit multiple branches both in *Phaenicia* (Fig. 2A,C) and in *Drosophila* (see Fig. 8 in Fischbach and Dittrich, 1989). Furthermore, whereas Tm1 is thought to be restricted to a single retinotopic column with its medulla arborizations arranged more or less symmetrically around the column axis, the corresponding arborizations of Tm9 are asymmetrical and clearly occupy two neighboring columns, with fine processes that appear to extend into a third column (Fig. 2B).

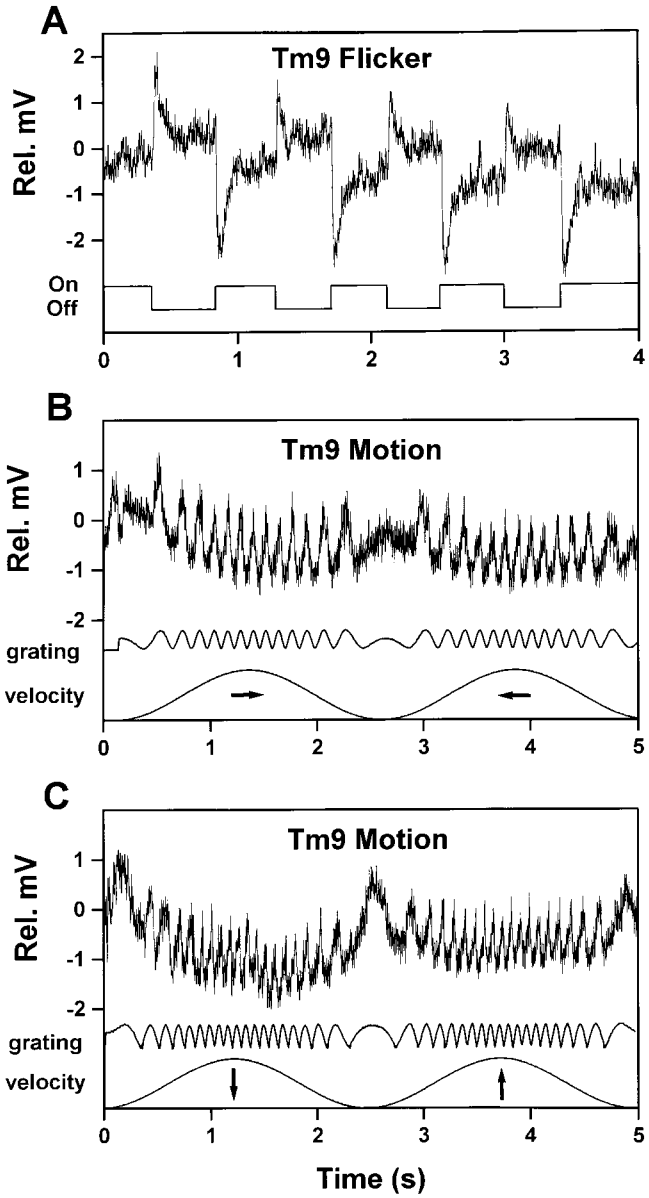
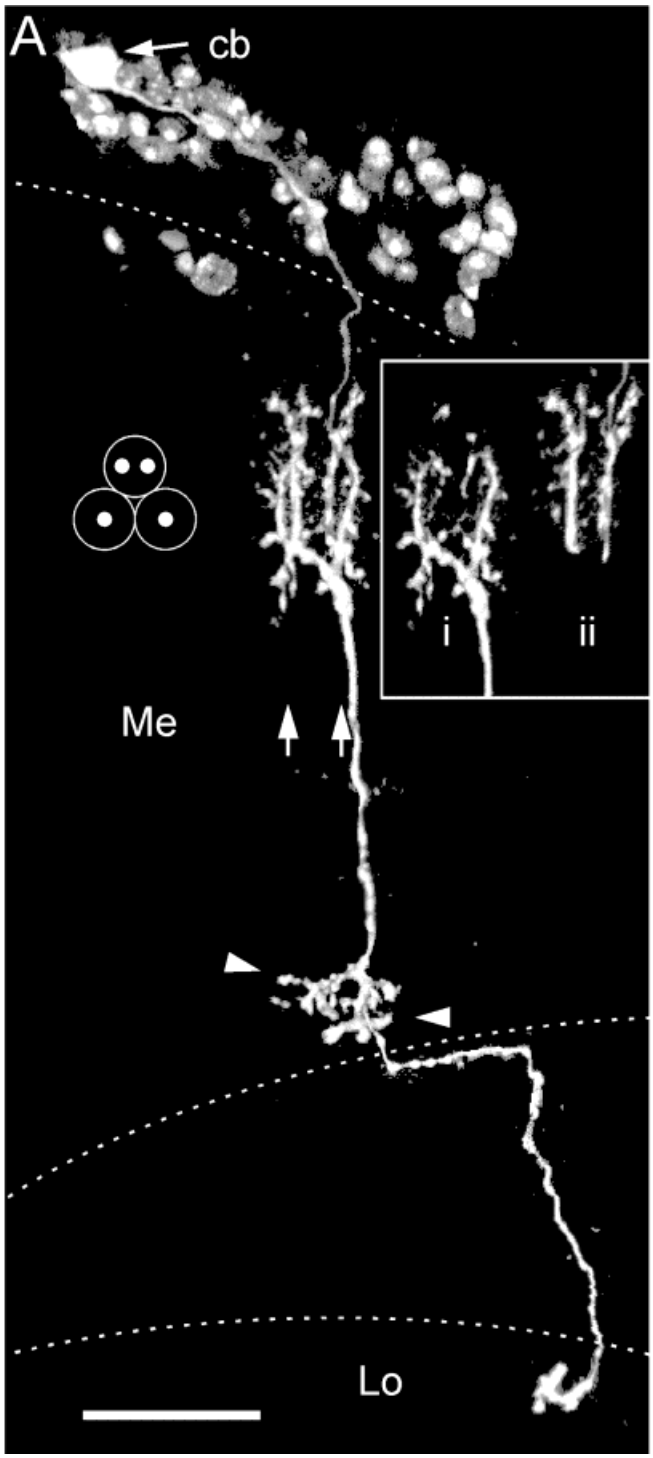


Fig. 12. **A–C:** Recordings from the type 9 transmedullary (Tm) cell (Tm9) illustrated in Figure 2A–C. Responses to computer-generated flicker (A) and motion at sinusoidally varied velocities (B, 0–70°/second; C, 0–93°/second) show no evidence for sensitivity to motion direction or contrast frequency.

The intracellular responses of Tm9 to flicker (Fig. 12A) were ON-hyperpolarizations and OFF-depolarizations, similar to those observed in a previous recording from Tm1 (Douglass and Strausfeld, 1995). Responses to motion,



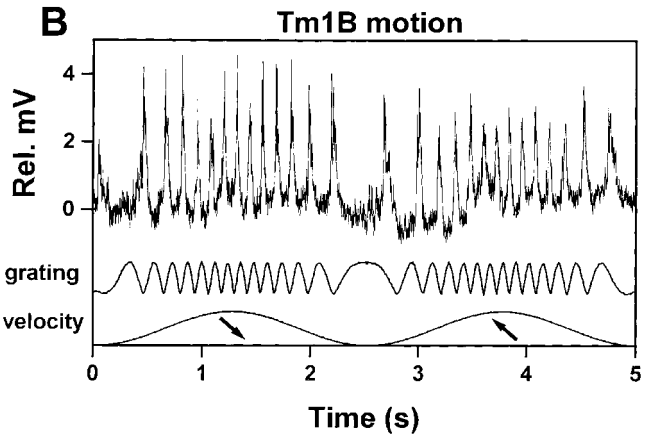


Fig. 13. **A:** Confocal reconstruction of a new type of transmedullary (Tm) cell, Tm1b, stained with Lucifer yellow. The preparation, as described in Figures 2A–C and 10, was counterstained with propidium iodide. Cell bodies in the chiasma between the medulla (Me) and the lobula (Lo) were omitted for clarity, as were most of the cell bodies distal to the medulla. Vertical arrows mark two adjacent retinotopic columns. In the outer medulla, there are four recurrent branches from the axon that appear to reside within three neighboring retinotopic columns (**left inset**; schematic cross section). Successive confocal projections (**right inset**) show two of these branches near the level of the axon (i) and two at a level shared with the cell body (cb) fiber (ii). Note that the axon collaterals in the proximal medulla (arrowheads) lie distal to the T4 layer (see Fig. 1), but here, they appear to overlap with this layer because the sectioning angle was oblique. **B:** Nondirectionally selective responses of Tm1b to motion with sinusoidally varied velocity (0–70°/second). Scale bar = 20 μm.

however, were clearly different in these two cells. Whereas the responses of Tm1 to grating motion were characterized by direction-specific changes in the frequency of voltage fluctuations, similar stimulation of Tm9 (Fig. 12B,C) produced fluctuations at the grating contrast frequency only, with no evidence for directional selectivity.

Type 1 B Tm cell (Tm1B). Figure 13 illustrates a heretofore unknown small-field Tm cell. Despite a short-lived recording that yielded only preliminary physiological information, this neuron is of tremendous interest because of its unique and striking morphology (see below). Upon initial impalement, Tm1b showed a stable resting membrane potential of −48 mV. Stimulation with wide-field, diagonal, grating motion and sinusoidally varied velocity (Fig. 13B) produced 3–4 mV depolarizations that followed the variable contrast frequency. The phase difference between the depolarizations and the record of grating motion cycles was roughly constant over the 0–12 Hz

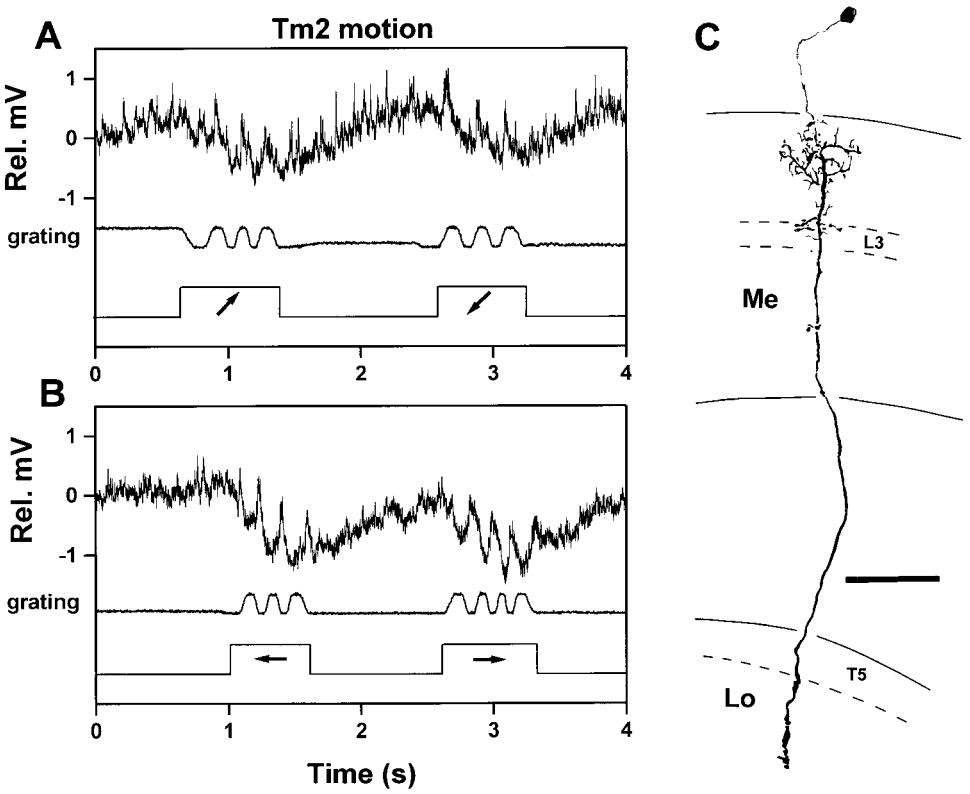


Fig. 14. **A–C:** Type 2 transmedullary (Tm) cell (Tm2). Nondirectionally selective responses to motion consisted of hyperpolarizations with superimposed fluctuations at the grating contrast frequency. Small background depolarizations (A) diminished rapidly (B). C: L3, medullary stratum (dashed lines) corresponding to the L3 monopolar cell terminals; T5, outer lobula stratum defined by dendrites of T5 neurons. Scale bar = 30 μm.

range of contrast frequencies presented, and the amplitudes of the depolarizations appeared to be independent of both speed and direction. The depolarizing responses, however, diminished rapidly to zero within the first minute of the recording, precluding additional tests at other motion directions or with flicker stimuli.

This neuron, like Tm9, appears superficially similar to Tm1, but closer examination reveals remarkable differences that are suggestive of important functional consequences. The similarities with Tm1 (Buschbeck and Strausfeld, 1996) include outer medulla dendritic arbors that are restricted mainly to the L2 layer, and axon collaterals deep within the medulla at layer M9 (just distal to the T4 dendritic layer). Tm1, however, has two main columnar trunks in the outer medulla, neither of which is thought to extend beyond the parent retinotopic column, whereas Tm1b has four main branches that occupy at least three columns (see below). In addition, the deep medulla axon collaterals of Tm1b branch profusely and reside in at least two adjacent columns. At this level, an axon collateral is relatively poorly developed in Tm1 and is entirely absent from Tm9.

The relationship between the four branches of Tm1b and the retinotopic architecture of the distal medulla was analyzed with three-dimensional reconstructions and confocal projections (not shown) in which the contrast and brightness of the neuropil background were enhanced (see, e.g., Fig. 2B). Each branch of the more widely spaced pair (Fig. 13A, right inset) is centered within its own retinotopic column (Fig. 13A, vertical arrows), whereas the second two branches are closer together, and thus appear to share a third column behind the first two. Three-dimensional reconstructions viewed at 90° from the sectioning plane support this model (Fig. 13A, schematic at left), because the distance between the two pairs of branches corresponds to the 6–7 μm interommatidial spacing at this level of the medulla.

Type 2 Tm neuron. Tm2 neurons have been described from *Drosophila* and *Musca* (Fischbach and Dittrich, 1989; Strausfeld, 1976). This is a small-field Tm neuron of distinctive appearance, with branches that span some four column diameters at the level of L2 LMC cell terminals, and with a terminal that extends into the lobula somewhat deeper than the T5 dendritic layer. A neuron was impaled briefly (Fig. 14) that is Tm2-like in its general morphology, but unlike previous descriptions, this cell lacks axon collaterals within the deep medulla. Although the quality of this recording deteriorated rapidly, it did provide some preliminary information regarding the response properties during stimulation with moving gratings. During the initial several seconds following impalement, the baseline activity when the projected grating stimulus remained stationary was dominated by small, transient depolarizations (Fig. 14A). Responses to motion were characterized by voltage fluctuations that matched the contrast frequency of the grating; these were superimposed on weak, sustained hyperpolarizations. No direction or orientation selectivity was evident, although the rapid deterioration of

the background transient depolarizations and the contrast-frequency fluctuations preclude a definitive conclusion in this regard. The presence of fluctuations at the contrast frequency suggests a maximum functional receptive field diameter not much larger than the 25° spatial wavelength of the grating, whereas the morphology of the outer medulla dendritic arbor sets a minimum field diameter of about two columns (ca. 3–5°).

DISCUSSION

Common afferent supply from the medulla to the lobula and lobula plate: Centripetal Y cells

Y cells comprise a diverse morphological class that includes many types of small dendritic field neurons, which probably occur in each retinotopic column, as well as a variety of wider field neurons that, although they are arranged retinotopically, occur one to every several columns in a manner similar to that of retinotopic neurons within the lobula complex. Given this diversity and the simple fact that Y cells connect three major visual processing neuropils, this class of interneurons must play crucial roles in visual discrimination and behavior.

Y17 cells or variants thereof have been identified previously in *Musca* (Strausfeld, 1976), *Sarcophaga* (Gilbert et al., 1991), and *Drosophila* (Fischbach and Dittrich, 1989; termed TmY9). The most striking feature of Y17 is its linear, tangential retinotopic architecture. The main tangential trunks that arise from the columnar axon within the medulla, lobula, and lobula plate all project in the same retinotopic direction, and finer arborizations are restricted to a small region around these processes. One consequence of this architecture is that the lobula plate terminals are restricted to a single stratum, unlike most such projections from columnar Y cells, which, instead, traverse the entire depth of the neuropil. In the current example from *Phaenicia* (Fig. 10), the tangential processes all reach in directions that correspond to the downward direction in their respective retinotopic maps, and the lobula plate projection lies within the vertically sensitive VS stratum of this neuropil. Previous accounts have illustrated each process of Y17 neurons reaching caudally (Fischbach and Dittrich, 1989) or anteriorly (Gilbert et al., 1991), with the lobula plate projection in the horizontally sensitive HS layer. Together, these observations suggest that there may be four morphological classes of Y17 cells, each with all of its tangential branches oriented upward, downward, anteriorly, or caudally with respect to the visual field. In addition, the limited evidence suggests that the depth of the lobula plate projection corresponds to the orientation of the tangential branches. If this is true, then these properties may be shared with T4 and T5 cells, small-field lobula plate afferents that are well known to segregate into four subpopulations terminating at four distinct lobula plate strata. Indeed, Y17-like asymmetries in the orientation of dendritic tree branches of T4 and T5 cells have been noted both in Lepidoptera (Strausfeld and Blest, 1970) and Diptera (Strausfeld, 1970; Buschbeck and Strausfeld, 1996), but they have not been correlated with the depths of their terminals.

The functional implications of the retinotopically polarized, tangential organization of Y17 are unknown. First, in common with all retinotopic Y cells, Y17's basic morphology seems well suited for spatial and temporal coordination of activity within the medulla, lobula, and lobula plate. In contrast with columnar Y neurons, the spatial asymmetry of the tangential branch in the medulla could provide Y17 with a morphological basis for directional sensitivity to moving edges, although experimental evidence shows that elementary motion detection is possible with interactions between elements that are separated by no more than a single visual sampling unit (Riehle and Franceschini, 1989). Moreover, in the only recording from Y17 to date, Gilbert et al. (1991) found no evidence for directional selectivity. An alternative suggestion is that neurons with single, long, thin, tangential branches can serve as elementary detectors of edge or bar orientation and position. The strongest responses to such stimuli would be expected when they are aligned with the branch. However, there are many other cell types within the medulla of the fly that meet these criteria without the added complication of having a similar retinotopic, tangential organization in three separate neuropils. The outer medulla arborizations of Y17 are in the M3 layer, a level that is shared with the terminals of R8 photoreceptors and fails to show 2-deoxyglucose activity labeling after motion stimulation (Bausenwein and Fischbach, 1992). These observations are suggestive of involvement in chromatic rather than motion-processing pathways.

The anatomical organization of Y1 (Fig. 11) is complementary to that of Y17, suggesting quite different functional roles. Instead of tangential processes restricted to just one or a few strata, Y1 has a small-field columnar organization in all three neuropils, but with arborizations at multiple levels. The projections of similar Y cells in the lobula complex have also been illustrated in a tabanid fly (Cajal and Sanchez, 1915). Y1 is reminiscent of neurons named TmY5 and TmY5a in *Drosophila* (Fischbach and Dittrich, 1989), but TmY5 exhibits some arborization within the serpentine layer, both TmY neurons lack axon collaterals within the outer one-quarter of the lobula, and their arborizations within the lobula plate are relatively sparse.

In the recording from Y1, the bar-motion tests showed clear responses to the presence of light within this cell's receptive field, but neither bar motion nor grating motion revealed any motion-specific response. What other functional roles are possible for Y1? The small-field organization of this neuron clearly suggests involvement in the processing of retinotopically mapped signals. Along with several other types of Y cells, the presence in Y1 of arborizations at various levels suggests interactions with several distinct retinotopic pathways, each of which may be specialized for processing specific, low-level visual features, including motion direction, object orientation, and chromatic properties (Strausfeld and Lee, 1991; O'Carroll, 1993). Thus, it is not surprising that Y1 responds to grating motion, bar motion, and flicker in a noisy and apparently nonspecific manner. This neuron may be more selective for completely different low-level visual parameters, such as wavelength or e-vector orientation. Alternatively, optimal stimuli may be those in which higher-level features of visual scenes interact (e.g., object orientation or color contrast).

Centrifugal retinotopic neurons from the lobula plate to the medulla

A handful of Y cells, including Y18 (Fig. 9) and CY2 (Douglass and Strausfeld, 1996; see Fig. 5), exhibit struc-

tural polarities that suggest centrifugal functional properties. In Y18 (see Fig. 1), the cell body behind the lobula plate is connected to processes deep within this neuropil. This arborization provides an axon that bifurcates to the lobula and medulla. The lobula terminal is varicose and sparsely branched, features that are associated with output terminals. Although the lobula plate and medulla arbors both show a mixture of spiny and varicose specializations, the physiological data are suggestive of major inputs from the lobula plate rather than the medulla. Y18 showed alternately hyperpolarizing and depolarizing responses to changes in motion direction, an expression of directional selectivity that is known in lobula plate neurons but not in the medulla (Douglass and Strausfeld, 1995). In addition, the robust sensitivity to vertical motion is consistent with inputs from the deeper lobula plate strata (Buchner et al., 1984), precisely where the arbors of this Y18 were localized.

Gilbert et al. (1991; see their Fig. 22) reported having stained and recorded from a neuron termed Y18 in *Sarcophaga* that, unlike its counterpart in *Phaenicia* (Fig. 9), exhibited exclusively hyperpolarizing responses to flicker and no directional selectivity to motion. Although both neurons are structurally centrifugal, their morphologies differ in other ways that may be related to the discrepancy in responses to directional motion. The lobula plate arborizations of the *Sarcophaga* Y18 appear to extend through all four of the directionally selective strata, whereas those of the present Y18 are restricted to the inner layer of the neuropil. In addition, the lobula component of the *Sarcophaga* Y18 is columnar, narrow, and penetrates deeply into this neuropil, whereas that of *Phaenicia* is distinctly bistratified and penetrates no more than one-third of the lobula. In these respects, the *Sarcophaga* Y18 resembles more closely the original description from *Musca* (Strausfeld, 1976). With respect to the medulla terminals, however, a distal concentration of globular varicosities is shared by the *Phaenicia* Y18 (Fig. 9) and the Y18 in *Musca* (Strausfeld, 1976) but was not noted in the example from *Sarcophaga* (Gilbert et al., 1991). Variations in terminal morphologies do not necessarily imply nonhomologous neurons, because differences may arise due to a common target neuron that has species-specific differences in dendritic stratification. In any case, the morphological differences between these two Y18 variants are clearly suggestive of functional differences in directional motion-processing properties.

Structural and functional variability among retinotopic medulla efferents

Y cells. Centripetal Y cells all share the same basic morphology: dendrites in the medulla and an axon that bifurcates to the lobula plate and medulla. Their cell bodies are located either above the medulla (and thus are similar to Tm cells that supply the lobula) or between the medulla and the lobula plate. Hitherto, Y cells have been classified mainly according to their dendritic arrangements in the medulla. However, comparisons of descriptions from Golgi studies and intracellular fills reveal differences that may have important functional implications.

Y17 in *Musca* (Strausfeld, 1976) and its possible homologue, TmY9, in *Drosophila* (Fischbach and Dittrich, 1989), for example, share a small axon collateral in the proximal medulla. This feature is also present in a variant termed Y10 in *Musca*, but has not yet been observed in *Phaenicia* or *Sarcophaga*. The lobula plate and lobula arborizations of the Y17 type are more variable still: In *Musca*, the lobula plate terminal is columnar rather than tangential; in *Drosophila*, *Musca*, and *Phaenicia*, respectively, the lobula terminals have one to three main branches. Because current information is generally limited to a single illustration from a given genus or family, it is unclear to what extent these variations reflect consistent taxon-specific differences, regional specializations across the retinotopic mosaic, or simply developmental variability or "noise" (Macagno et al., 1973). However, in the case of the three superficially similar Tm neurons that have been documented in Calliphoridae and that terminate at the T5 dendritic layer of the outer lobula (Tm1, Tm1b, and Tm9), the substantial anatomical and physiological differences we have noted indicate their identities as distinct Tm types.

Proposed roles of Tm9, Tm1b, and Tm1 in motion processing. Because Tm9, Tm1b, and Tm1 represent distinct classes of Tm neurons that all terminate at the level of T5 dendrites in the lobula, it is important to consider whether the anatomical differences can help explain physiological properties. Among these three small-field Tm types, directionally selective responses to motion have been recorded only from Tm1; this and other lines of evidence suggest that Tm1 is a crucial element of an elementary motion-detection circuit (Douglass and Strausfeld, 1995). A very basic requirement for directionally sensitive elementary motion detection is that two neighboring inputs be integrated in some manner. Thus, it may seem paradoxical that Tm1 should be directionally selective, with outer medulla arborizations that appear to be restricted to a single retinotopic column (Strausfeld, 1976), whereas Tm9 and Tm1b appear to be insensitive to motion direction, yet have branched aborizations that extend beyond the parent column. This "paradox" is resolved mainly by the fact that the required neighboring inputs need not arrive intracellularly. Moreover, both modeling and physiological studies indicate that nonlinear, synaptic interactions are crucial at the integration stage (for reviews, see Egelhaaf and Borst, 1993; Grzywacz et al., 1995). Such synaptic interactions are easiest to envisage for Tm1, and are likely to involve L2 lamina monopolar cell terminals (Bausenwein and Fischbach, 1992; Buschbeck and Strausfeld, 1996). With regard to Tm1b, an additional morphological feature may weaken further its suitability for directional motion processing: The trapezoidal arrangement of its four columnar branches in the outer medulla (Fig. 13) provides no clear morphological basis for a preferred/null directional axis. In conclusion, Tm1 remains the best candidate Tm type for participation in directional motion processing. Tm1b and Tm9 may mediate other, as yet unidentified, local interactions among retinotopic columns.

Intrinsic neurons in the lobula plate and lobula complex

Cells that are intrinsic to the lobula complex are little known and poorly understood, as noted above (see Results). In Diptera, Golgi studies in the lamina and medulla have identified intrinsic neurons that, like horizontal cells in the vertebrate retina, connect local areas of the retinotopic mosaic via tangential axons (Strausfeld, 1970; Campos-Ortega and Strausfeld, 1973). In addition, there are

four examples of tangential neurons that are localized to the lobula complex (Fig. 3 in Strausfeld, 1970; Fig. 14 in Speck and Strausfeld, 1983; Figs. 5 and 7 in Fischbach and Dittrich, 1989; Fig. 8, present study). Two of these are intrinsic to the lobula plate, and two others have an additional tangential projection within the lobula. Although no clear input or output region has been identified in the intracellularly recorded, amacrine-like LPL neuron described here, its directional selectivity (Fig. 8) suggests that it receives major inputs from the second stratum of the HS layer, which is associated with regressive motion (Buchner et al., 1984).

Properties of retinotopic efferent pathways originating in the lobula complex

The present account provides further evidence that visual channels from the retina segregate into three major parallel systems (see Strausfeld and Gilbert, 1992): medulla efferents to the lobula plate, efferents to the lobula, and Y cells that innervate both of these neuropils. Within each major system, evidence for additional subsystems with distinct functional roles is beginning to emerge. It is not yet known how many Y cells respond selectively to motion, because recordings have been obtained in only a few representatives of this abundant cell type. Nevertheless, of those sampled, only the centrifugal Y cells respond unambiguously to directional motion (CY2 in Douglass and Strausfeld, 1996; Y18, present study, Fig. 9), suggesting that activity in the lobula plate is reported to the medulla and to the lobula. The few centripetal Y cells sampled here and in another account (Gilbert et al., 1991) do not reveal clear directional sensitivity, yet they have shown subtle differences in responses to motion vs. flicker. A further indication that Y cells are involved in motion processing is provided by their lobula plate morphology: In common with T4 and T5 neurons, the axon collaterals to the lobula plate of many centripetal Y cells provide stratified arrangements of specializations, the levels of which correspond to directional motion-sensitive layers of this neuropil (Buchner et al., 1984). The additional presence of amacrine cells and local motion-sensitive interneurons within the lobula plate further suggests that this neuropil does not merely collate information about directional motion through its wide-field tangentials, but that its complex cellular and synaptic arrangements are involved in the integration of motion with other features of the visual world.

Thus, LLP and LPL neurons, the efferents shared by the lobula plate and the lobula, are attractive candidates for pathways that encode information about local and other features associated with visual movement stimuli, such as contrast frequency (Fig. 7, type 4 LLP) and object position or shape (Fig. 6, type 1 LPL). Thus far, recordings from these retinotopic lobula complex efferents suggest that LLP and LPL neurons themselves might be subdivisible into two major functional classes of retinotopic output. Neurons equipped with wide dendritic fields in the lobula plate (e.g., that shown in Fig. 3) can have more pronounced directionally selective properties than neurons with small dendritic fields. If the latter neurons are indicative of a distinct functional class, then they may encode complex receptive field properties, such as those suggested by the type 1 LPL (Fig. 6) in which responses to visual motion depend on the location of the motion stimulus within the physiological receptive field of the neuron. Other complex types of responses to motion are suggested by the small-field LPL neuron shown in Figures 4 and 5, which may discriminate wide-field flicker from motion. Future research will test the validity of these generalizations.

Recordings from Tm cells that project to deeper layers of the lobula (Gilbert et al., 1991; present study, Fig. 14) suggest an absence of directional selectivity. This finding is consistent with the possible roles of lobula output neurons in form or color processing and with the hypothesis that they are segregated from retinotopic pathways that are involved in processing motion and other features of the visual world. A more complete evaluation of parallel organization in the insect optic lobes (Strausfeld and Lee, 1991) awaits the results of additional recordings from lobula neurons and from neurons that are shared by both neuropils of the lobula complex.

In conclusion, the present results demonstrate that intracellular physiological studies of the insect visual system can be broadened to include the smallest optic lobe efferents and their medullary supply. These species of neurons are the most diverse in the insect visual system, and are likely to provide the organism with the kinds of sophisticated visual properties suggested by recent studies on form and pattern discrimination (O'Carroll, 1993; Srinivasan et al., 1993; Dafni et al., 1997; Yang and Maddess, 1997).

ACKNOWLEDGMENTS

We thank Robert Gomez, B.S., for technical and photographic assistance; Lars Hansen, B.A., for his expertise in graphics processing and composition; and Patty Jansma for assistance with confocal microscopy.

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