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Visual Motion Detection Circuits in Flies: Peripheral Motion Computation by Identified Small-Field Retinotopic Neurons

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Giant motion-sensitive tangential neurons in the lobula plate are thought to be cardinal elements in the oculomotor pathways of flies. However, these large neurons do not themselves compute motion, and elementary motion detectors have been proposed only from theory. Here we identify the forms, projections, and responses of small-field retinotopic neurons that comprise a well known pathway from the retina to the lobula plate. Already at the level of the second and third synapses beneath the photoreceptor layer, certain of these small elements show responses that distinguish motion from flicker. At a level equivalent to the vertebrate inner plexiform layer (the fly's outer medulla) at least one retinotopic element is directionally selective. At the inner medulla, small retinotopic neurons with bushy dendrites extending through a few neighboring columns leave the inner medulla and supply inputs onto lobula plate tangentials. These medulla relays have directionally selective responses that are indistinguishable from those of large-field tangentials except for their amplitude and modulation with contrast frequency. Centrifugal neurons leading back from the inner medulla out to the lamina also show orientation-selective responses to motion. The results suggest that specific cell types between the lamina and inner medulla correspond to stages of the Hassenstein–Reichardt correlation model of motion detection.

[Key words: insects, vision, early motion computation, retinotopic pathways, theoretical pathways, identified neurons]

The detection of motion is an essential first step in processing the visual information that guides much of animal behavior. In insects as in vertebrates (Land, 1982), motion detection underlies optokinetic head and body movements, figure-surround discrimination, depth perception by binocular or motion stereopsis, and visual equilibrium provided by the detection of visual flow fields and their angular velocities (Hausen, 1982a,b; Egelhaaf et al., 1988; Hengstenberg, 1993). Detailed predictions about the properties of motion detection circuits have been obtained from behavioral, electrophysiological, and theoretical investigations (Torre and Poggio, 1978; Egelhaaf and Borst, 1993). Nonetheless, the identities of actual nerve cells that might comprise an elementary motion detection (emd) circuit remain almost entirely unknown in arthropods even though transmission between the primary photoreceptors and large first-order interneurons (lamina monopolar cells, LMCs) is well documented (Laughlin, 1989), as are the responses of large tangential neurons deep within the system, at the level of the lobula plate (Hausen and Egelhaaf, 1989). Between these levels there intervenes the medulla, a complex neuropil comprising many thousands of relay neurons and amacrine cells (Strausfeld, 1976). This article addresses the functional organization within these levels.

In the vertebrate retina bipolar cells, ganglion cells, and displaced amacrines have been strongly implicated in elementary motion computation (Maturana and Frank, 1963; Barlow and Levick, 1965; Werblin et al., 1988) but, apart from in the rabbit retina (Masland and Tauchi, 1986) where starburst amacrines provide a dual transmitter mechanism for the on and off responses of motion-sensitive ganglion cells, the exact cellular mechanisms of vertebrate emds are still incompletely understood, and directional selectivity may arise *de novo* in the primate visual cortex (Hildreth and Koch, 1987). In insects, although there is a wealth of valuable experimental information on elementary motion detection, this information is mainly indirect, relying on the ability to sequentially stimulate neighboring visual sampling points and record the effects such stimuli have on the activity of lobula plate neurons that are postsynaptic to the presumed motion detector circuits (Franceschini et al., 1989).

This article presents one of two complementary approaches to elucidating the cellular nature of emd circuits in insects. The first approach (Buschbeck and Strausfeld, 1994; Buschbeck, unpublished observations) involves comparative anatomical studies which recognize classes of small retinotopic neurons that are ubiquitous to the Diptera, and have analogous arrangements in other insect taxa. The existence of an evolutionarily conserved subset of small-field retinotopic neurons leading to the lobula plate is characteristic of an achromatic magnocellular optomotor pathway involving few synapses between the photoreceptors and motion-sensitive collator neurons in the lobula plate (Strausfeld and Lee, 1991). The morphological characteristics of certain cell types and the layer relationships among these cells suggest that they may play a crucial and universal role in the processing of motion and/or other elementary categories of visual information. The present account describes the combined intracellular electrophysiological and anatomical properties of certain of these conserved cell types, focusing on the extent to which identified cells can encode parameters of motion stimuli. All of these cells reside either wholly or partially in the medulla, and are anatom-

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ically small-field, retinotopically mapped, and well known from anatomical studies (Strausfeld, 1976; Strausfeld and Nässel, 1980; Strausfeld and Lee, 1991; Buschbeck and Strausfeld, 1994; Buschbeck, unpublished observations). Until recently (Gilbert et al., 1991), the only available physiological information on motion responses in the dipteran medulla consisted of recordings from unidentified cells (Mimura, 1971, 1974; Arnett, 1972; DeVoe, 1980; Osorio, 1992).

Numerous studies have demonstrated the motion, orientation, and directionally selective responses of lobula plate tangential neurons, including VS, HS, FD (figure detection), and other tangentials, such as those that extend heterolaterally between the optic lobes and deutocerebrum (for review, see Hausen and Egelhaaf, 1989). Theoretical studies, based on observations of the optomotor responses and on intracellular recordings from the lobula plate tangentials, have relied exclusively on Reichardt-Hassenstein-type emd circuits as the input devices. The original correlation model for motion detection (Hassenstein and Reichardt, 1956) was proposed specifically for insects, and its essential features have withstood a number of experimental tests involving insect vision (for review, see Hildreth and Koch, 1987; Borst and Egelhaaf, 1989; Franceschini et al., 1989). Actual emd circuits provided by connections among real neurons cannot of course be expected to match all features of a model, and the existing data are still too incomplete for the construction of a detailed circuit (cf. Getting, 1989). The current results nevertheless permit the association of individual cells with specific conceptual stages of motion computation, and thus suggest the preliminary outlines of a real motion-detecting pathway in the fly brain.

Materials and Methods

Experiments employed two species of calliphorid flies, *Phaenicia sericata* and *Phormia regina*, both obtained from laboratory colonies maintained on fresh liver (for larvae) and sugar, water, and vitamin-enriched powdered milk (adults). Intracellular recordings were obtained during visual stimulation with flicker and motion. Recorded cells were stained, and their histological profiles examined with confocal and conventional epifluorescence microscopy.

Electrophysiological recordings. Intact male or female flies were inserted in small plastic tubes with the head and anterior thorax protruding from either end. Low-melting-point wax was used to fix the thorax to the tube, immobilize the head at a 45° angle from the horizontal axis of the fly, and fix the proboscis in a protruded position in order to reduce muscular movements near the brain. A piece of cuticle was removed from either the left or right posterior side of the head, the exposed tissue was bathed in insect saline (after O'Shea and Adams, 1981; buffered with 5.0 mM TES and adjusted to pH 7.2), and sufficient fatty tissue and tracheal material were removed to expose selected portions of the optic lobes (lamina, medulla, or lobula plate). To access the chiasma, between the lamina and medulla, an additional narrow strip of tissue was removed from the dorsal rim of the compound eye. The recording pipette was inserted into the rear surface of the optic lobes with a Leitz micromanipulator. The amplifier's capacitative override switch was often used to assist in penetrations. Apart from the VS cell, neurons described here are of exceptionally small diameter, ranging from 2.5 μm down to less than 1 μm. Neurons are also extremely densely packed and can occasionally be unintentionally filled en passant with Lucifer yellow, resulting in multiple fills that require careful measurements in the tissue so as to reconstruct the passage of the electrode and thus determine the order in which cells were encountered, with the last (and usually most intense) neuron corresponding to the electrophysiological record. Measures taken to minimize ambiguities included (1) keeping detailed voice records during experiments, (2) usually impaling each brain with a single pipette advanced in only one direction, and (3) concluding experiments as soon as one recording of interest had been obtained. Thus, if two cells were stained but did not contact each other, they could be matched with the electrophysiological data according to their depth in the brain. Borosilicate pipettes (Sutter Instruments BF100-78-10, Novato, CA) were fabricated on a laser puller (Sutter model P-2000), filled with 4% Lucifer yellow in distilled water (Molecular Probes L-453, Eugene, OR), backfilled with 0.1 M LiCl (Sigma, St. Louis, MO), and connected to a standard AgCl electrode. The passive electrode was connected to the saline bathing the head via a broken-tipped pipette. *In situ* resistances typically ranged from circa 70 to 140 MΩ, as measured with the intracellular amplifier (AM Neuroprobe 1600, Everett, WA). Intracellular membrane voltages were recorded on VCR tape with a PCM adapter (Vetter 3000A, ca. 6 kHz effective sampling rate) and/or digitized and stored at 10 kHz on a personal computer equipped with data acquisition hardware and software (Datawave Inc., Longmont, CO). Since most responses were nonspiking and consisted of relatively slow DC shifts in membrane potential, data were converted (via 100 point averaging) to an effective sampling frequency of 100 Hz, except where noted in Figures 3 and 4.

Stimuli. Impaled cells typically were stimulated first with motion, then flicker, followed by additional motion stimuli when possible. Visual motion stimuli were produced by projecting, via a front surface mirror, high-contrast photographic images of a 1-D square-wave grating onto a circular rear-projection screen constructed from velum drawing paper and framed with black construction paper. All experiments were performed in a darkened room, so that visual input to the fly came directly from the projection screen. The spatial and temporal frequencies employed (see below) are known to represent effective motion stimuli for lobula plate tangential cells (e.g., Hausen, 1982b; Hengstenberg, 1982).

The grating pattern was illuminated with a tungsten–halogen fiber-optic source, and the pattern was positioned in the rear focal plane of a small projection lens with a micromanipulator. The manipulator was also used to manually move the pattern, typically at a temporal frequency of circa 4 Hz. All mechanical movements took place outside of a Faraday cage in which the fly was mounted on a pedestal, with the front of the head 2.0 cm from the center of the 55 mm diameter projection screen. The screen thus presented a 108° field of view. A horizontal grating was used to produce vertical movements, and a vertical grating for horizontal movements. Diagonal movements were simulated with horizontal and vertical movements of gratings oriented at 45° from horizontal. The grating images displayed straight edges and high-contrast stripes. Thus, the illusion of diagonal movement was effective for human observers, and presumably for the flies. The grating spatial wavelength was circa 0.04 cpd. The directions of grating movements were recorded with potentiometers connected to the moving parts of the manipulator, and a silicon photodiode (United Detector Technology PIN 10DP, Hawthorne, CA) was used to monitor the grating speed. A beamsplitter located behind the projection screen was used to reflect a low-intensity image of the grating onto the photodiode. The diode was situated in the focal plane of the grating image, and positioned at the center of the field of view.

A wide-field flicker stimulus was produced in either of the following ways. (1) Using white light at 63 lux, the evenly illuminated projection screen was interrupted by a black cardboard shutter out of the focal plane of the projection lens, and monitored by the photodiode. (2) Using green light (at 4 lux; see below), an array of 40 high-intensity green LEDs (Gilway Technical Lamp E166, Woburn, MA) with a typical emission peak at 563 nm (half-bandwidth 25 nm) was directed at the projection screen and powered with a function generator operated in square-wave mode (Beckman Industrial FG2, Brea, CA). Flicker intensity was nearly uniform over the entire surface of the projection screen (variation <0.1 log unit). In order to facilitate comparisons between responses to flicker and motion, the two types of stimulus were adjusted to similar intensities, contrast ratios, and spectral compositions. For the LED flicker stimulus, this was accomplished by placing a pair of colored lucite filters in the optical path of the grating stimulus, and adjusting the projected intensities of flicker ON and OFF to match the bright and dark stripe intensities of the grating pattern. The mean intensities of the green flicker and grating stimuli were circa 4 lux, with contrast ratios greater than 15. The combined transmission spectrum of the lucite filters (measured on a Gilford scanning spectrophotometer) was maximal at 560 nm, with a half-bandwidth of 32 nm. The spectral maxima of the flicker and motion stimuli were considerably greater than the visual pigment absorption maxima for the R1–R6 photoreceptors (360 and 490 nm; Hardie, 1979). Green LEDs were selected, however, because of their much greater brightness compared with shorter-wave-

length LEDs. The neurons VS, T5 #1, L5, and iTm were tested using white light; all others were tested using green light.

Histology and anatomical reconstructions. Cells were stained with Lucifer yellow by applying a steady hyperpolarizing current (1–3 nA) for from 30 sec to several minutes. Brains were promptly fixed for 1–2 hr at room temperature in 4% Mallinkrodt formalin buffered with Sorensen's, Millonig's, or cacodylate buffer at a pH of 6.9–7.2 (Strausfeld et al., 1983). Unless records were made from the lamina, first chiasma, or outer medulla, the retina and ommatidia were removed immediately in order to prevent the fluorescent ommochromes from diffusing into the neural tissue. Some preparations were then fixed overnight at 4°. Fixed tissue was rinsed twice in buffer, dehydrated in an ethanol series followed by acetone, embedded in Spurr's (1969), and sectioned transversely at 14 μm on a sliding microtome. After recording from the lamina or first chiasma, in order not to damage their neurons, some retina was left attached. In these cases ommochrome fluorescence interfered with confocal study, and the identity of lamina-associated neurons relied on their morphology in the medulla, for which there is a volume of reference data from Golgi studies (Strausfeld, 1976; Buschbeck and Strausfeld, 1994; Buschbeck, unpublished observations). Profiles of stained cells (optical sections) were photographed every 0.5–1 μm with a confocal epifluorescence microscope (Bio-Rad MRC 600), or serially photographed onto Kodak Ektachrome 400 at 1 μm intervals on a Leitz Aristoplan epifluorescence microscope, and reconstructed by projecting images from a front surface mirror.

Results

Intracellular recordings and Lucifer yellow injections were performed in motion- and/or flicker-sensitive cells which had projections to the lamina, medulla, lobula plate, and T5 dendrite layer of the lobula, a displaced layer of the medulla (Strausfeld and Lee, 1991). Initial resting membrane potentials ranged from circa −10 to −60 mV. The most stable recordings were continued for 10–20 min; others lasted for only 1–2 min before responses to stimulation ceased. The presentation of results in this article begins with what might be considered a finished product of elementary motion detection, a recording from a nonspiking wide-field vertically sensitive (VS) tangential cell in the lobula plate. From this point some four synapses beyond the photoreceptor level, we will proceed distally to the lamina, where its large monopolar cells (LMCs) receive direct inputs from photoreceptors, and which has, to date, usually been thought not to participate in motion detection. Figure 1 shows the overall organization of the achromatic magnocellular pathway from the lamina to the lobula plate, and illustrates several of the small-field, motion-responsive cells to be described below.

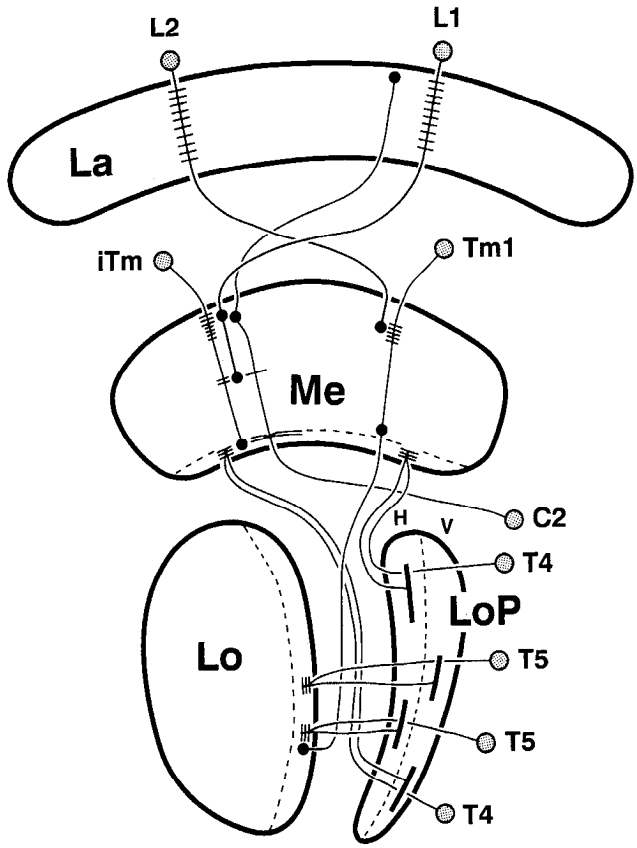

Figure 1. Schematic summary of magnocellular optomotor pathways in the calliphorid fly brain, showing anatomical relationships among small-field retinotopic neurons (*stippled circles,* cell bodies). Ipsilateral optic lobes (*La,* lamina; *Me,* medulla; *Lo,* lobula; *LoP,* lobula plate) are shown in horizontal section, with anterior to the left. Dendrites (*thin bars*) of large monopolar cells L1 and L2 receive achromatic inputs from photoreceptors R1–6 (not shown), and terminate (*filled circles*) at characteristic levels (outer L1; L2; inner L1) that coincide with dendrites of transmedullary cells iTm and Tm1. iTm terminates at the T4 dendritic layer in the deep medulla; Tm1 has terminals distal to the T4 layer and in the T5 dendritic layer of the outer lobula. T4 and T5 terminate (*thick bars*) in the lobula plate on wide-field tangential cells (not shown) sensitive to horizontal (*H*) and vertical (*V*) motion. Also shown is a centrifugal feedback cell, C2. See Results and Discussion for additional details. Based on Strausfeld (1989), Strausfeld and Lee (1991), and Buschbeck and Strausfeld (1994, unpublished observations).

Direction-selective small-field inputs to giant motion-sensitive neurons

Figure 2*A* illustrates a recording during wide-field horizontal and vertical motion from the third of a row of 11 giant VS (vertically sensitive) neurons in the lobula plate (Fig. 2*B*). Responses were encoded as directionally selective DC shifts in the membrane potential. The strongest depolarizations occurred during downward motion, while motion in the opposite (null) direction produced hyperpolarizations. These responses have been well documented for VS neurons in *Calliphora* (Hengstenberg, 1982), and also typify responses to horizontal motion recorded from the orthogonal set of giant lobula plate tangentials, the horizontal neurons (HS; Hausen, 1982a,b). As shown by electron microscopy of Golgi-impregnated neurons, the dendrites of VS neurons are supplied by synapses from the terminals of bushy T-cells (Strausfeld and Lee, 1991), T4 and T5, the dendrites of which originate, respectively, in the innermost stratum of the medulla and the outermost stratum of the lobula (Fig. 1). T4 and T5, respectively, are visited by the terminals of the intrinsic transmedullary neuron "iTm" and the long-axoned transmedullary projection neuron "Tm1" (Strausfeld and Lee, 1991).

We have succeeded in recording from and staining two T5 cells. In the first (Fig. 2*C,D*), light microscopy identified the electrode's point of entry in the axon, about 5 μm beneath its first dendritic branch. In this T5 recording (Fig. 2*C*), the responses closely resembled the DC shifts that characterize HS and VS cells, but with considerably smaller potential fluctuations. The recorded T5 neuron depolarized to progressive motion and hyperpolarized to regressive motion. Notably, unlike the responses of VS and HS neurons (see Fig. 2*A*; Hausen, 1982a,b; Hengstenberg, 1982), but expected of its predicted small receptive field (Strausfeld and Lee, 1991), T5 responses included a component that is phase locked to the moving stimulus, with the membrane potential fluctuating at the contrast frequency of the grating, particularly during progressive (front-to-back over the

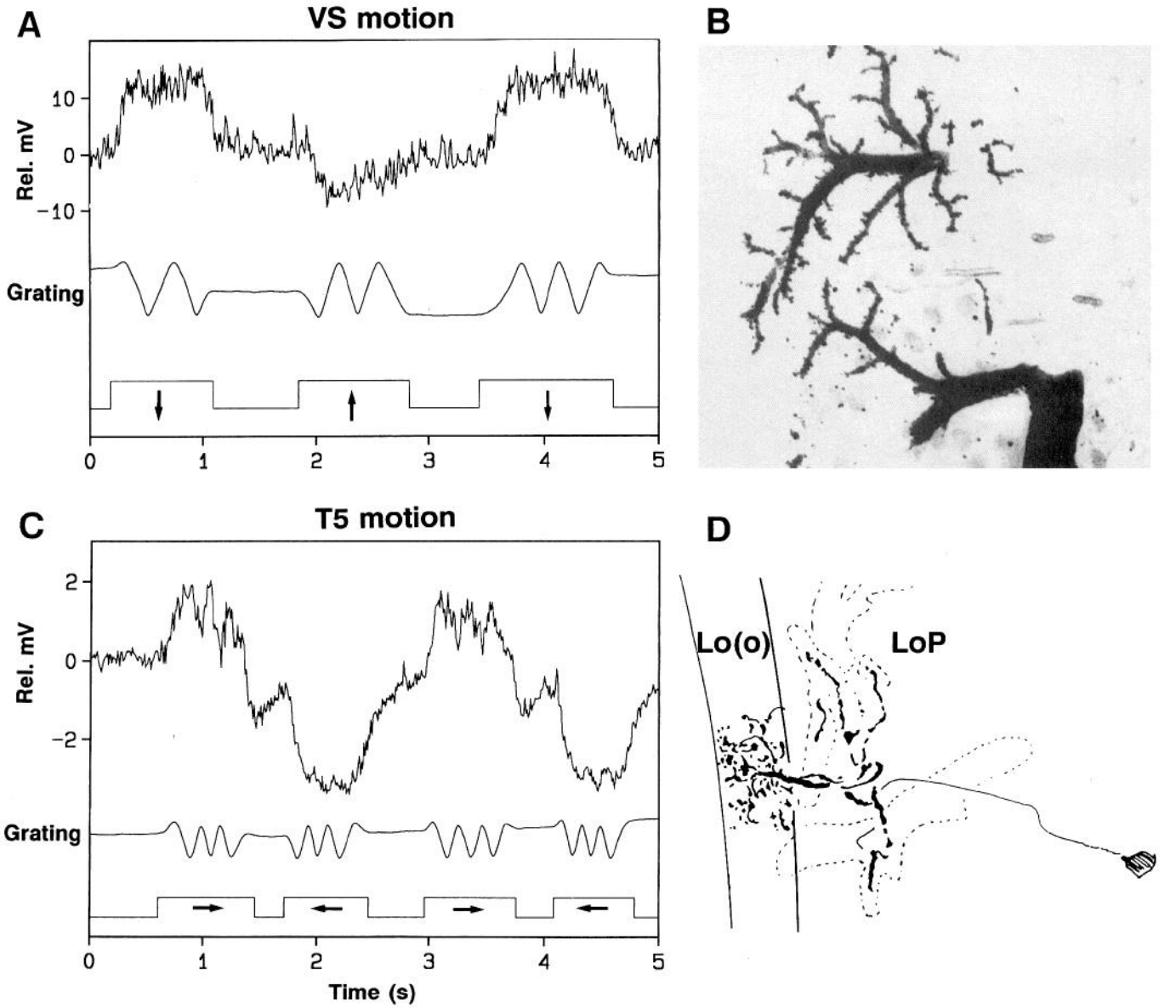

Figure 2. Intracellular responses to motion and anatomical reconstructions from a VS (*A, B*) and a T5 cell (*C, D*). In *A* and *C, upper traces* show intracellular voltage relative to the prestimulus baseline (*0*), the *middle trace* records grating movements monitored with the photodiode, and the *lower trace* indicates the timing, stimulus duration, and direction (*arrows*) of grating motion. *B,* Confocal projection (negative image) of a portion of the VS cell in *A*. Dorsal is up. *D,* Reconstruction of T5 from 1 μm serial optical sections of the plastic-embedded Lucifer-filled neuron, recorded as color transparencies. *Lo P,* lobula plate; *Lo(o),* outer lobula stratum (terminal area of medullary Tm 1 neurons). The dark nonautofluorescent profiles of an HS cell are indicated by *dashed lines.* Magnification: *B,* 320×; *D,* 400×.

retina) motion. Responses to both flicker and motion were recorded from a second T5 cell (Fig. 3). In this case, however, light microscopy suggested penetration was into the base of one of its larger dendrites which was particularly brightly stained, and showed damage suggestive of impalement. The ON response to flicker (Fig. 3*A*) consisted of a transient hyperpolarization followed by a weaker, sustained hyperpolarization; OFF responses were transient depolarizations with no sustained component. Responses to motion were directionally selective, but instead of DC shifts, responses were manifested as changes in the frequency of miniature excitatory potentials (meps; Fig. 3*B*). This activity was clearly audible on the audio monitor during the recording accompanied by lower-frequency fluctuations in the membrane potential. Since the latter fluctuations appeared unrelated to stimuli, they were removed by subtracting a 15 point (7.5 msec) moving average from the data. Figure 3*B* illustrates an excerpt from the filtered data, which were used to obtain a directional selectivity plot (Fig. 3*C*) by computing the numbers of pre- and poststimulus depolarizations that exceeded an arbitrary threshold (0.2 mV) above the baseline. Maximal excitation in T5 was elicited by progressive motion directed slightly upwards (mean vector angle = 25°). Moreover, whereas progressive motion elicited trains of meps (Fig. 3*Bi*), motion in the opposite direction inhibited spontaneous activity (Fig. 3*Bii*). This recording demonstrates direction-dependent excitation and inhibition at the T5 dendritic tree and is in accordance with the direction-dependent DC shifts shown from axonal recordings of another T5 (Fig. 2*B*).

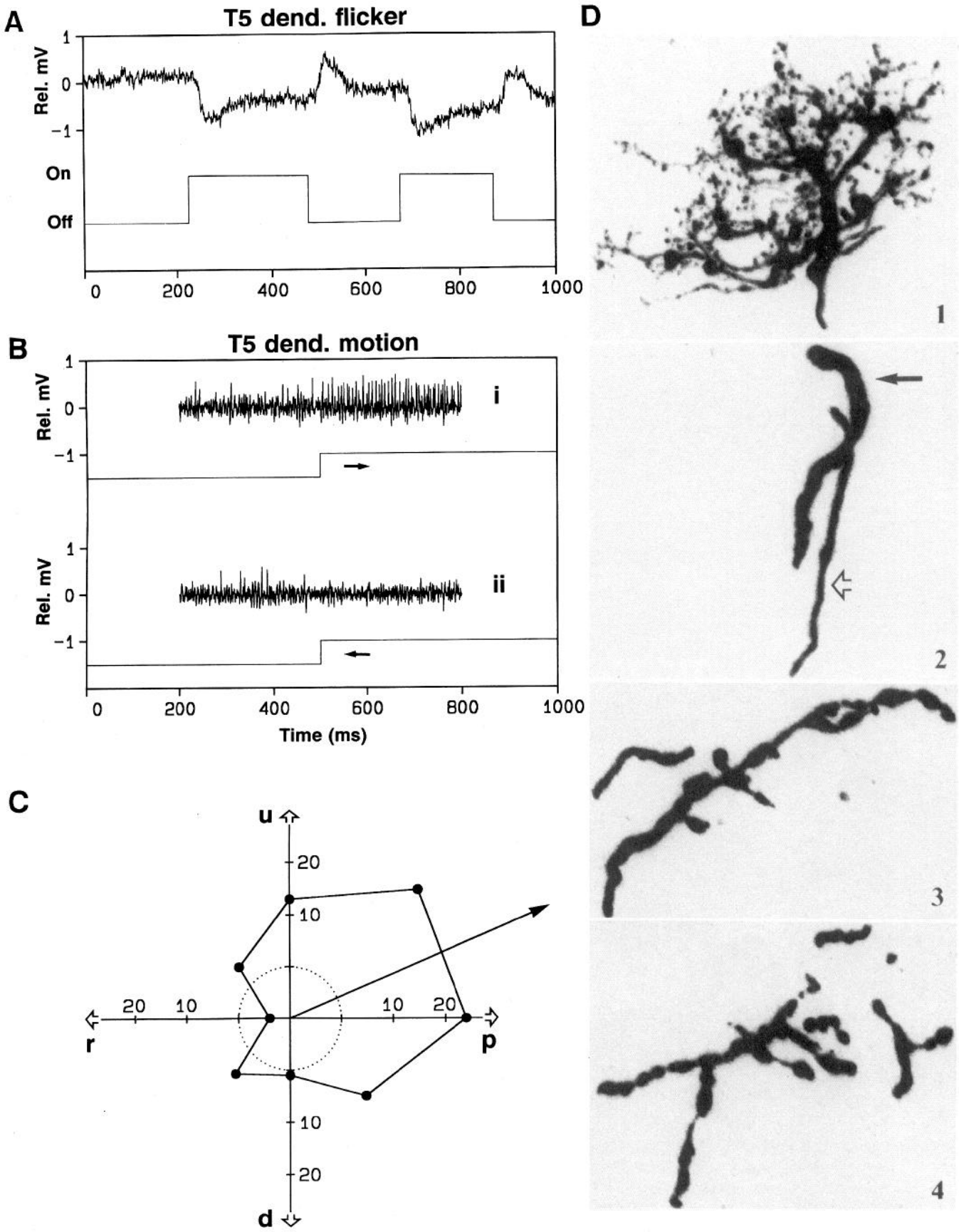

Figure 3. Flicker and motion responses in a dendritic recording from T5. *A,* Flicker responses: hyperpolarizations to ON; transient depolarizations to OFF. *Upper trace* shows intracellular voltage relative to baseline; *lower trace* shows flicker ON and OFF. Plotted at an effective sampling

Retinotopic motion-sensitive and -insensitive neurons target direction-selective small-field neurons

The transmedullary cells iTm and Tm1 are almost certainly the small-field inputs to T4 and T5, respectively (Strausfeld and Lee, 1991), and comparative studies have demonstrated that the dendrites of these T-cells and the terminals of Tm1 and iTm always coincide in depth, irrespective of species (Buschbeck and Strausfeld, 1994; Buschbeck, unpublished observations). The question, then, is whether afferents to the T4 and T5 neurons are already motion and directionally sensitive or if these properties are computed at the T4 or T5 dendrites. As in the T5 responses to flicker, both iTm and Tm1 exhibited hyperpolarizing ON responses (Figs. 4*A*, 5*A*) followed by a sustained plateau, and depolarizing OFF responses with no sustained component. Both cells also responded to motion, but in quite different ways. As illustrated for upward and downward motion in Figure 4*B*, iTm responded with gradually increasing hyperpolarizations and showed no evidence of selectivity for any of the eight standard motion directions. Tm1, however (Fig. 5*B*), responded with increased membrane potential fluctuations, and both the frequency and amplitude of these fluctuations were direction dependent. Responses to regressive (rightward) and progressive (leftward) movement differed in two ways. First, regressive motion produced higher-amplitude fluctuations overall. Second, whereas the major frequency components of responses to progressive motion were near the contrast frequency of the grating, responses to regressive motion included significant fluctuations at approximately twice the contrast frequency. These differences are discernible in individual traces (Fig. 5*B*), and are also evident in power spectral density functions averaged from the first four stimulus presentations (Fig. 5*C*). After the first several seconds of stimulation, responses to motion became increasingly hyperpolarizing and less directionally selective. Thus, it was not possible to analyze the overall directional selectivity of this cell.

How peripheral is motion sensitivity?

At what level neurons are first sensitive to motion remains a central question (Coombe et al., 1989). Responses of Tm1 suggest that it integrates motion information, possibly gained via its afferents from the lamina or via connections that exist between retinotopic columns at deeper levels in the medulla. Information about the representation of visual motion at these depths can be acquired by recording from centrifugal neurons that extend back into the lamina. In the outer medulla, at the level of Tm 1 dendrites, the type 1 lamina tangential cell (Lam tan 1; Strausfeld, 1976) has wide-field dendrites at the level of LMCs (Fig. 6*C*). Deep in the medulla, the type 2 lamina centrifugal cell (C2; Strausfeld, 1976) has dendrites at the level of the T4 neurons and a second layer at the level of the deep endings of the L1 monopolar cell (Fig. 7*C*). At both levels, C2 dendrites extend through a vertical domain of columns, an organization that is conserved across the Diptera (Buschbeck and Strausfeld, 1994).

The responses of Lam tan 1 and C2 differed in interesting ways. Lam tan 1 responded to flicker with small but otherwise typical ON and OFF responses (Fig. 6*A*, upper trace), but was completely silent in response to motion. In contrast, C2 showed a stronger response to flicker (Fig. 7*A*; especially the ON responses), and was hyperpolarized during motion (Fig. 7*B*). In addition, C2 exhibited small fluctuations in membrane potential at the contrast frequency. Significantly, these fluctuations were clearly evident during horizontal but not vertical motion, while responses to opposite directions of motion were indistinguishable. Thus C2 provides an orientation-selective motion-sensitive feedback to the outer stratum of the lamina where, in each retinotopic column, a C2 is presynaptic onto the necks of the L1, L2 cells, distal to the level at which they receive presynaptic inputs from the R1–R6 photoreceptors (Strausfeld and Nässel, 1980).

A significant feature of C2 is that this cell links adjacent medulla columns. Observations of Golgi-impregnated C2 neurons (N. J. Strausfeld, unpublished observations) have suggested that their axons ascend through one column, originating from the T4 dendritic layer, and then, at the medulla's outer surface, provide a distal swelling that appears to reach to the adjacent column. Confocal images convincingly demonstrated this important feature of C2. Although the medulla portion of the C2 axon lies almost entirely within a single column, it makes a short detour into an adjacent column (Fig. 7*C*, inset) where a characteristic swelling is placed to abut the incoming axons of L1 and L2. Interestingly, C2 does not stain with antibodies against GABA at this level, whereas the C2 terminals in the lamina do, suggesting two levels of presynaptic interaction by C2, mediated by two different transmitters (see Discussion).

Small interneurons associated with motion-insensitive afferents

Six centripetal channels originate from each retinotopic column (optic cartridge) in the lamina and project across the first optic chiasma into the medulla. The six cell types are the monopolar cells L1–L5 and the T1 centripetal cell (Strausfeld and Ortega, 1977). There are considerable data on the large monopolar cells (LMCs) L1–L3. LMCs are postsynaptic to photoreceptors R1–R6 (for review, see Strausfeld and Nässel, 1980), and have a wide working range (Laughlin, 1989). They respond biphasically to light-ON and light-OFF, inverting the sign of the photoreceptors, and do not discriminate motion from flicker (for reviews, see Laughlin, 1981, 1989). LMCs project across the first optic chiasma and terminate at two levels in the medulla where they are enwrapped by the dendrites of Tm1 and iTm (Strausfeld and Lee, 1991). In our study, we recorded from T1 and the two small monopolars (SMCs) L4 and L5. None of these is primarily postsynaptic to photoreceptors but each obtains its inputs in the lamina through intermediate neurons. The T1 (basket) neuron (Fig. 8*C*) is well known from light and electron microscopy of the laminas of calliphorid flies and *Drosophila melanogaster*. Just beneath the lamina, the T1 axon provides a coronet of six branches that ascend around a column of photoreceptor termi-

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frequency of 1 kHz. *B*, Example of intracellular responses (*upper traces*) to progressive (*i*), from front to back of the right eye, and regressive (*ii*) horizontal motion (*lower traces*); sampling frequency of 2 kHz. See Results for additional details. *C*, Polar plot of directional selectivity, calculated from the difference between the number of threshold crossings during (t = 500–800 msec in *B*), and prior to (t = 200–500 msec), stimulation with motion. *Dashed circle* shows the zero response level, where there is no difference in the rate of threshold crossings during and prior to stimulation. *Arrow* shows vector sum of the individual responses to eight directions of motion. *D*, Confocal projections (negative images) of (*1*) the dendritic region in the lobula (*2*), the axon (*solid arrow*) and cell body fiber (*open arrow*), and (*3, 4*) terminals in the lobula plate. Magnification in *D*, 1500×.

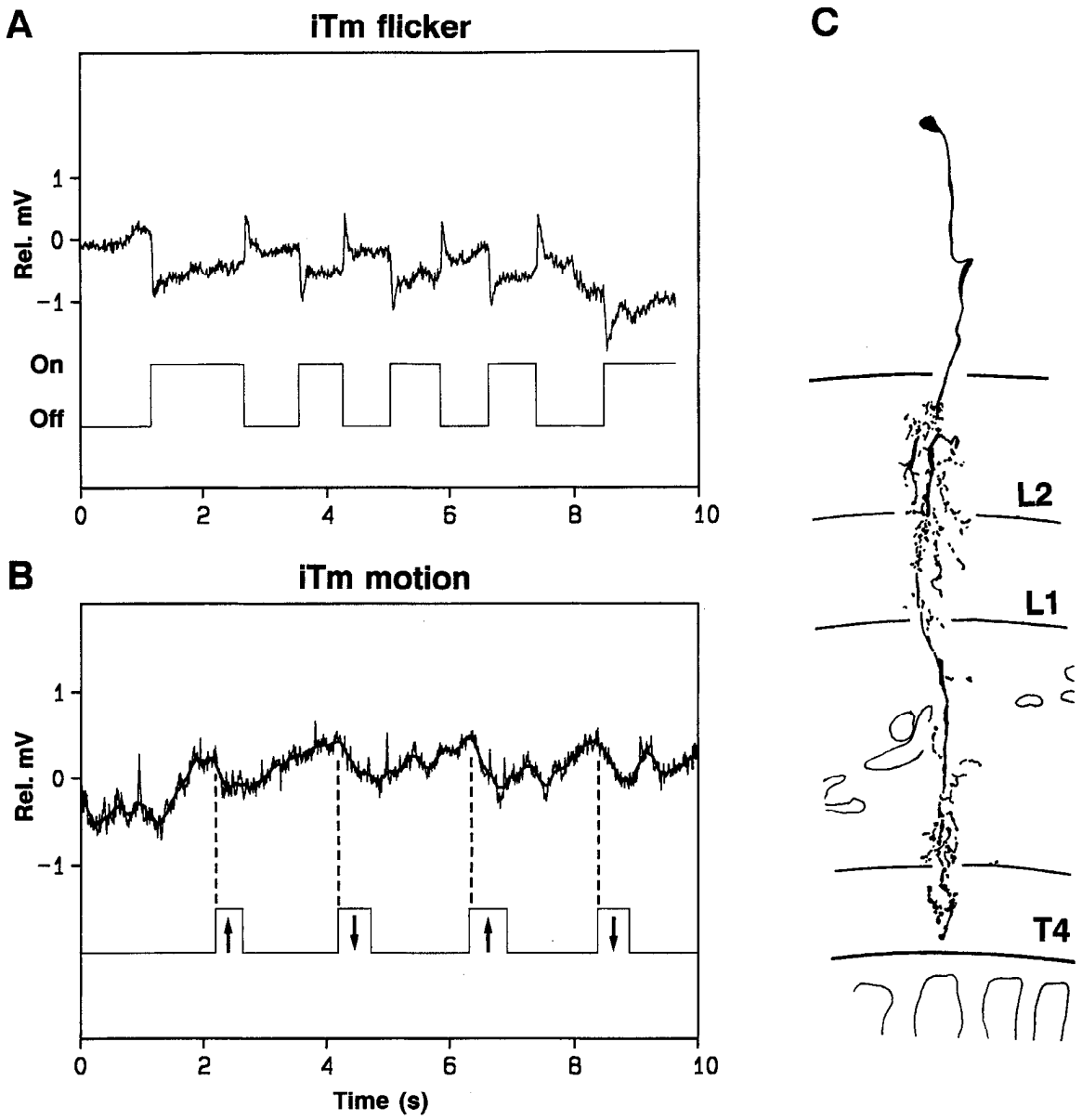

Figure 4. Flicker (*A*) and motion (*B*) responses in the intrinsic medullary neuron iTm. In *B*, 21 point moving average (*thick line*) is superimposed on the raw motion responses, and stimulus onset has been extended (*dotted lines*) to demonstrate onset of increasing hyperpolarization during stimulation. *C*, Anatomical reconstruction from fluorescence micrographs. *Horizontal lines* indicate the medullary strata that correspond to terminal specializations of L1, L2, and the dendrites of the bushy T-cell, T4. Magnification, 670×.

nals. T1 is postsynaptic to the lamina amacrine cells, themselves postsynaptic to the photoreceptor axons (Campos-Ortega and Strausfeld, 1973). T1 is thus one synapse removed from the primary photoreceptor input and, because the lamina amacrine embraces several cartridges, could be in a position to respond to inputs from several visual sampling points, or from a very narrow acceptance angle in the event that amacrine cells mediate lateral inhibition. An earlier account showed evidence that the acceptance angle of T1 was significantly smaller than that of single photoreceptors (Järvilehto and Zettler, 1973). Our recordings have focused on the responses of T1 to flicker and motion. Intracellular recordings exhibited the usual overall pattern of responses to flicker (see above), but response amplitudes (particularly to OFF) were weak (Fig. 8*A*). Motion stimuli (Fig. 8*B*) produced hyperpolarizing fluctuations at the contrast frequency, but these fluctuations were even weaker than the flicker responses, lacking a depolarizing component, and showed no evidence of directional selectivity. A confocal reconstruction of T1's projections into the L1 layer of the medulla (Fig. 8*C*) reveals a dense, delicately branched morphology.

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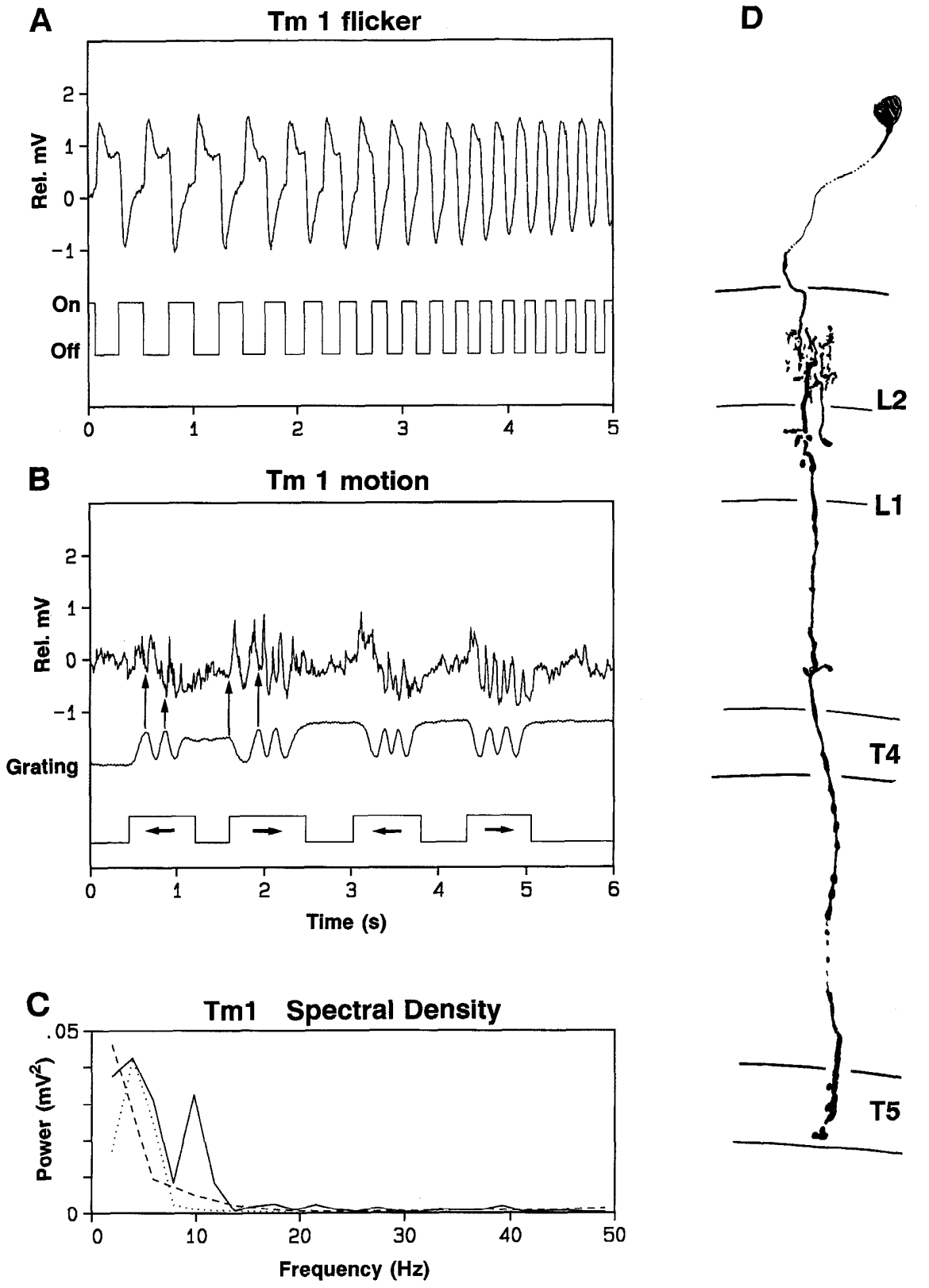
Figure 5. Responses to increasing flicker frequency (*A*) and to regressive and progressive horizontal motion (*B*) in the transmedullary cell Tm1. In *B*, *paired vertical arrows* illustrate individual cycles of grating motion, for which there are differences in the frequency of membrane potential



fluctuations during regressive (rightward) and progressive motion (*middle trace*). *C*, Power spectra illustrating direction-dependent change in frequency content of responses to motion. Mean spectra obtained from membrane potential fluctuations during regressive (*dashed lines*; $n = 4$) and progressive (*solid lines*; $n = 4$) motion are shown with the normalized mean power spectrum from grating motion (*dotted lines*; $n = 8$). Individual spectra were computed from digitized 4096 point records replayed from the first 512 msec of stimulation, and exhibited no significant power above 50 Hz. *D*, Reconstruction of the recorded Lucifer yellow–filled Tm1 from serial photomicrographs. L2, L1, and T4 layers of the medulla and the T5 layer in the outer lobula are indicated as reference points. Magnification, 670×.

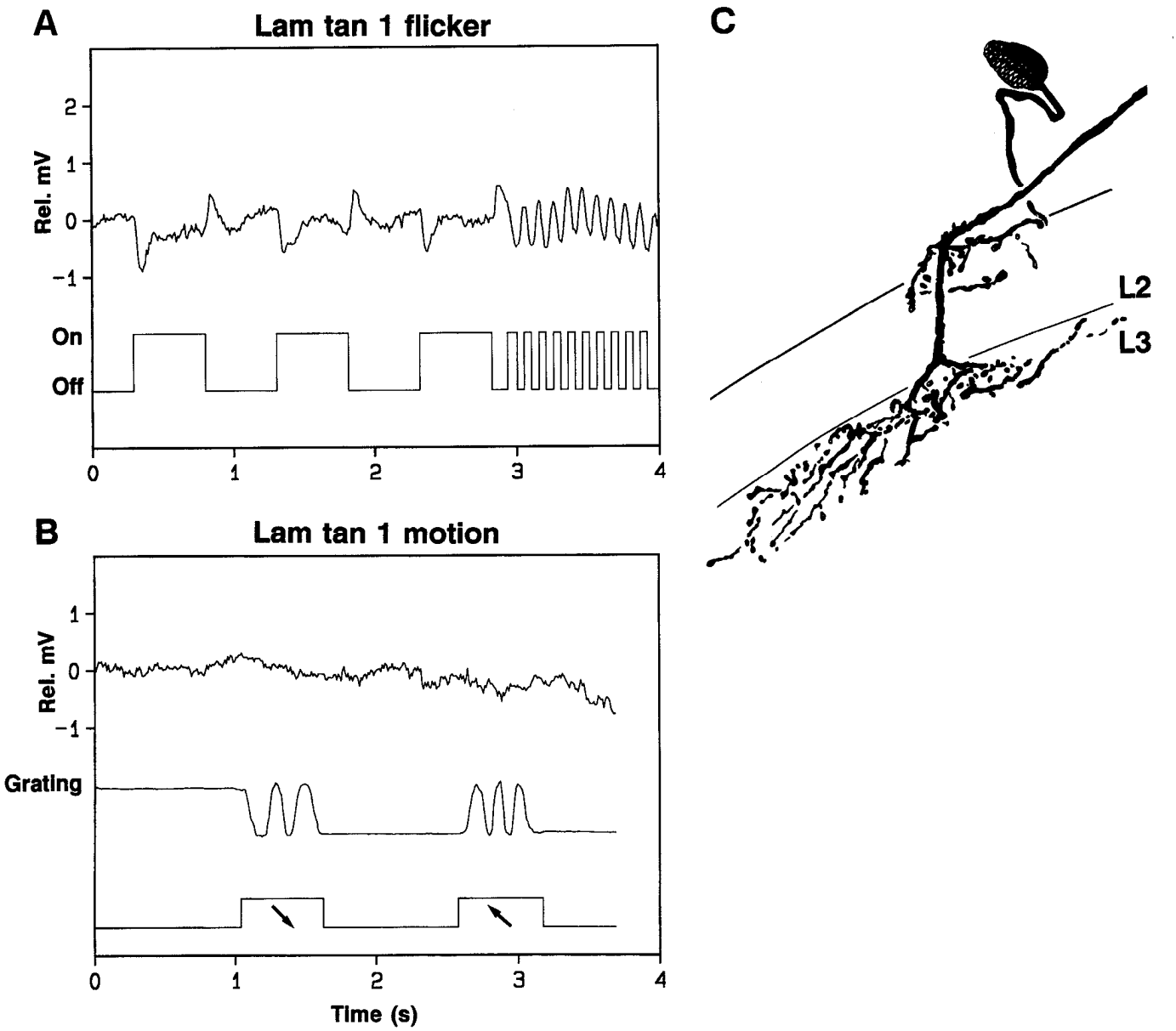


Figure 6. The type 1 lamina tangential cell's responses to slow and fast flicker (*A*) and its complete silence in response to diagonal motion (*B*). *C*, Reconstruction of Lam tan1 from serial photographs of five confocal stacks over a depth through the tissue of about 100 μm (the approximate diameter of its deeper dendritic field). The L2 and L3 layers of the medulla are indicated. Magnification, 600×.

The two SMCs from each cartridge have unusual axonal relationships with the L1 and L2 LMCs. L4 provides collaterals onto the axons of L1 and L2 in adjacent retinotopic columns, both in the lamina and in the medulla (Strausfeld and Campos-Ortega, 1973), and L5 terminals insinuate between the swollen endings of L1 and L2 and the dendrites of the transmedullary cells Tm1 and iTm (Strausfeld and Lee, 1991). Because its axon collaterals at the inner face of the lamina provide a rectilinear network among all the optic cartridges (Strausfeld and Braitenberg, 1970), L4 has figured in numerous debates about its possible role in motion computation (e.g., Braitenberg, 1970).

The flicker responses of the small monopolar cells L4 and L5 were fairly typical of other small-field retinotopic neurons (Figs. 9*A*, 10*A*), but L5 exhibited stronger responses to ON than to OFF. An important finding was that both cells were specifically affected by motion (Figs. 9*B*, 10*B*), but in different ways. In response to horizontal motion, L4 (Fig. 9*B*) exhibited mainly depolarizations which coincided with the stimulus contrast frequency, and showed direction-related phase shifts relative to the photodiode record. L5 (Fig. 10*B*), like C2, showed hyperpolarizing responses to motion with additional very small fluctuations at the contrast frequency. However, in contrast with C2, there was no suggestion of directional selectivity.

Discussion

Nonspiking interneurons

None of the cells described by us shows typical spiking responses, and it is therefore important to compare our results with those obtained from previous intracellular studies. Here we show intracellular recordings from several anatomically identified neu-

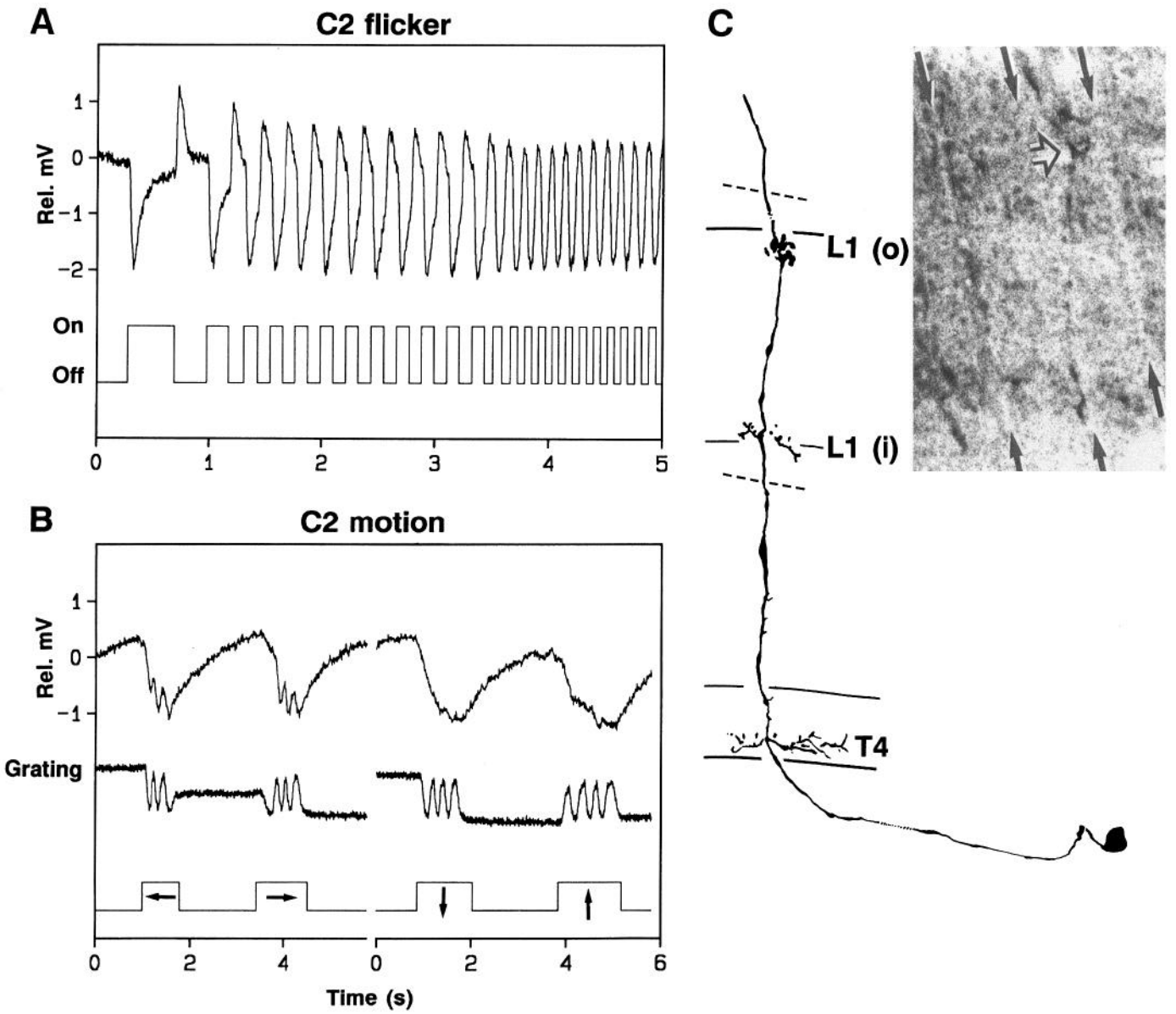


Figure 7. The C2 retinotopic centrifugal cell. Transient de- and hyperpolarizing responses to flicker (*A*) are distinguished from responses to horizontal and vertical motion (*B*) in which phase-locked modulations of the hyperpolarizing response are specifically elicited by horizontal motion. The reconstruction (*C*) indicates the T4 layer and the outer (*o*) and inner (*i*) layers corresponding to the two levels of L1 specializations (see Buschbeck and Strausfeld, 1994). *Inset* (region enclosed by *dashed lines* in reconstruction), Confocal projection of a lateral detour (at *open arrow*) by the C2 varicosity to the immediately adjacent column as C2 exits the outer medulla surface en route for the lamina. *Solid arrows* indicate the parent column of this C2 neuron and two neighboring columns. Magnification, 670× (*inset*, 920×).

rons that play potentially important roles in the early processing of motion. Most of these cells have not been previously identified in conjunction with recordings, and none except the VS cell has been shown to respond to motion. We demonstrate that computation of movement is already manifested by neurons in the deep medulla and its displaced (T5-Tm1 terminal) layer in the outer lobula. Small-field direction- or orientation-selective neurons project centripetally into the lobula plate (T5) or extend centrifugally out to the lamina (C2). However, even distal to these layers, certain other cells, such as Tm1, iTm, L4, and T1, show responses that are elicited by motion, as opposed to flicker, and certain of these are orientation selective as well.

How well do our recordings correspond to those obtained in previous studies? Only two cell types, T1 and VS neurons, provide direct comparison (Järvilehto and Zettler, 1973; Hengstenberg, 1982). The T1 centripetal neuron is known from a single recording of its nonspiking flicker responses in *Calliphora erythrocephala.* T1 responses we observed were consistent with these previous recordings. Previous recordings from VS neurons (Eckert and Bishop, 1978; Hengstenberg, 1982) showed that even these very large nerve cells conduct by mechanisms that do not conform to classical spikes. Our recordings from VS neurons concur. Among the remaining cells described here, responses to motion took various forms, including DC shifts in membrane potential, phasic fluctuations temporally correlated with the grating frequency, and small changes in background membrane potential.

Although extracellular studies have recorded spiking activity

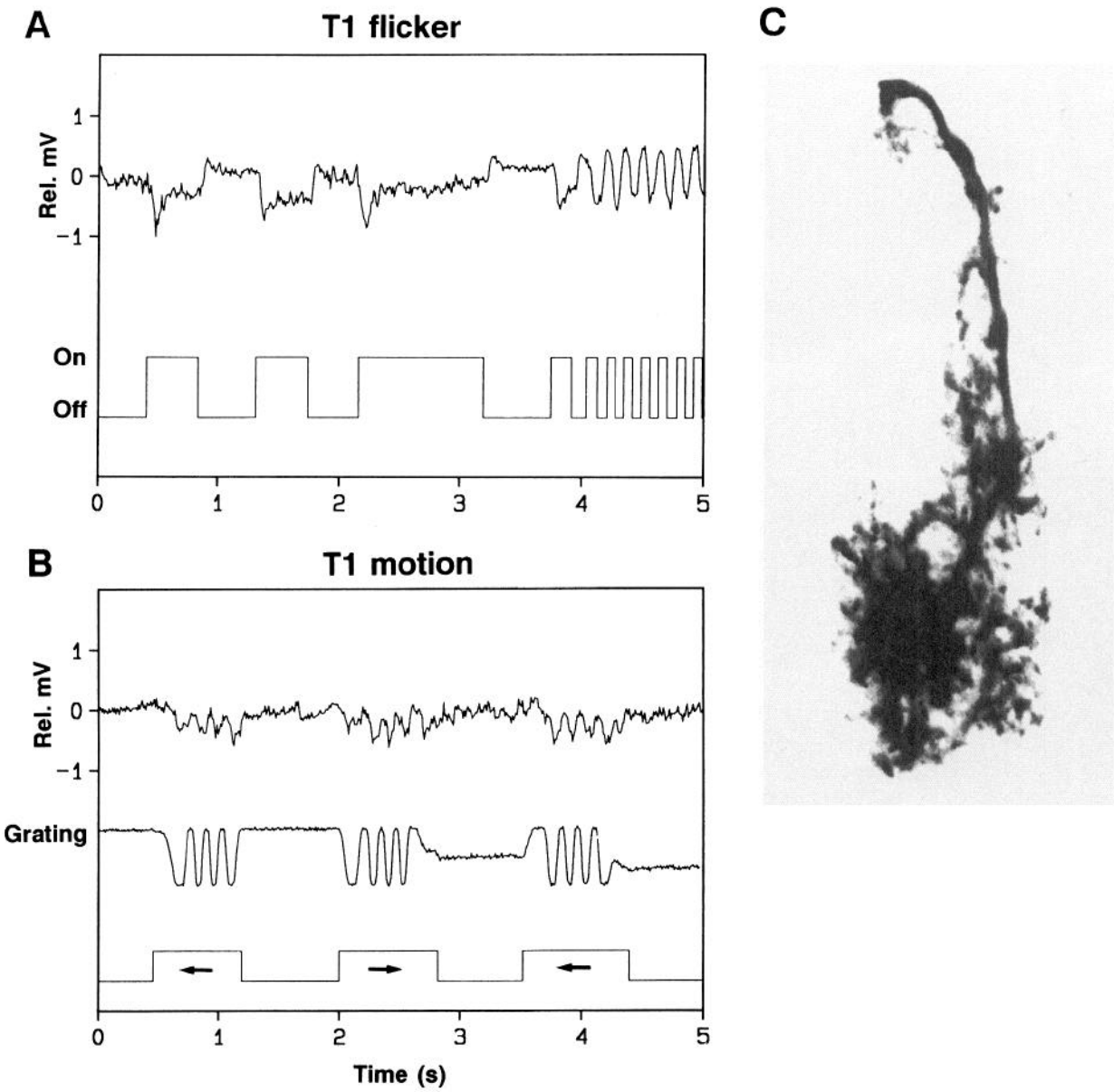


Figure 8. Biphasic transient responses to flicker (*A*) by the T1 centripetal neuron. Responses to motion show weak hyperpolarizations at the grating frequency and no depolarizations above baseline. *C,* Confocal reconstruction (negative image) of the T1 arborizations at the L2 layer of the medulla. Magnification, 2000×.

within fly optic lobes (Arnett, 1972; Osorio, 1992), the only convincing evidence for classical all-or-nothing responses in identified nerve cells has been obtained from intracellular studies of long-axoned neurons that link the lobula complex with the central or contralateral brain (Hausen and Egelhaaf, 1989; Gilbert and Strausfeld, 1992; Strausfeld et al., 1995). Whereas Hardie (1978, cited by Laughlin, 1984) reported spiking in the only previous recording to identify L4, we observed no spikes in this cell and are therefore unable to confirm Shaw's (1981) proposal that L4 corresponds to a narrow-field sustained spiking unit described by Arnett (1972). In general, nonspiking activity is fairly typical of small-field retinotopic elements of the medulla and lamina (DeVoe, 1980; Laughlin, 1981; Shaw, 1989), and of the lobula and lobula plate (Gilbert and Strausfeld, 1992). The flicker responses recorded in this study are similar to previous recordings from lamina monopolar cells L1 and L2 (Järvilehto and Zettler, 1971, 1973; Laughlin, 1989). All cells exhibited a hyperpolarization to ON followed by a sustained hyperpolarizing plateau, and a phasic depolarization to OFF. Response amplitudes were small, due to the relatively low stimulus intensities employed by us. Although flicker response latencies were not investigated in detail, they also fell within a range (ca. 15–20 msec) reported by Järvilehto and Zettler (1971) for LMCs stimulated at low intensities.

Cell identity

The anatomical identifications of our recorded cells correspond well with Golgi studies of neurons that are known to be retinotopically organized (Strausfeld, 1976). We have used a standard method of serially photographing Lucifer-stained cells for manual reconstruction using conventional epifluorescence microscopy; but, because lamina and medulla retinotopic neurons are

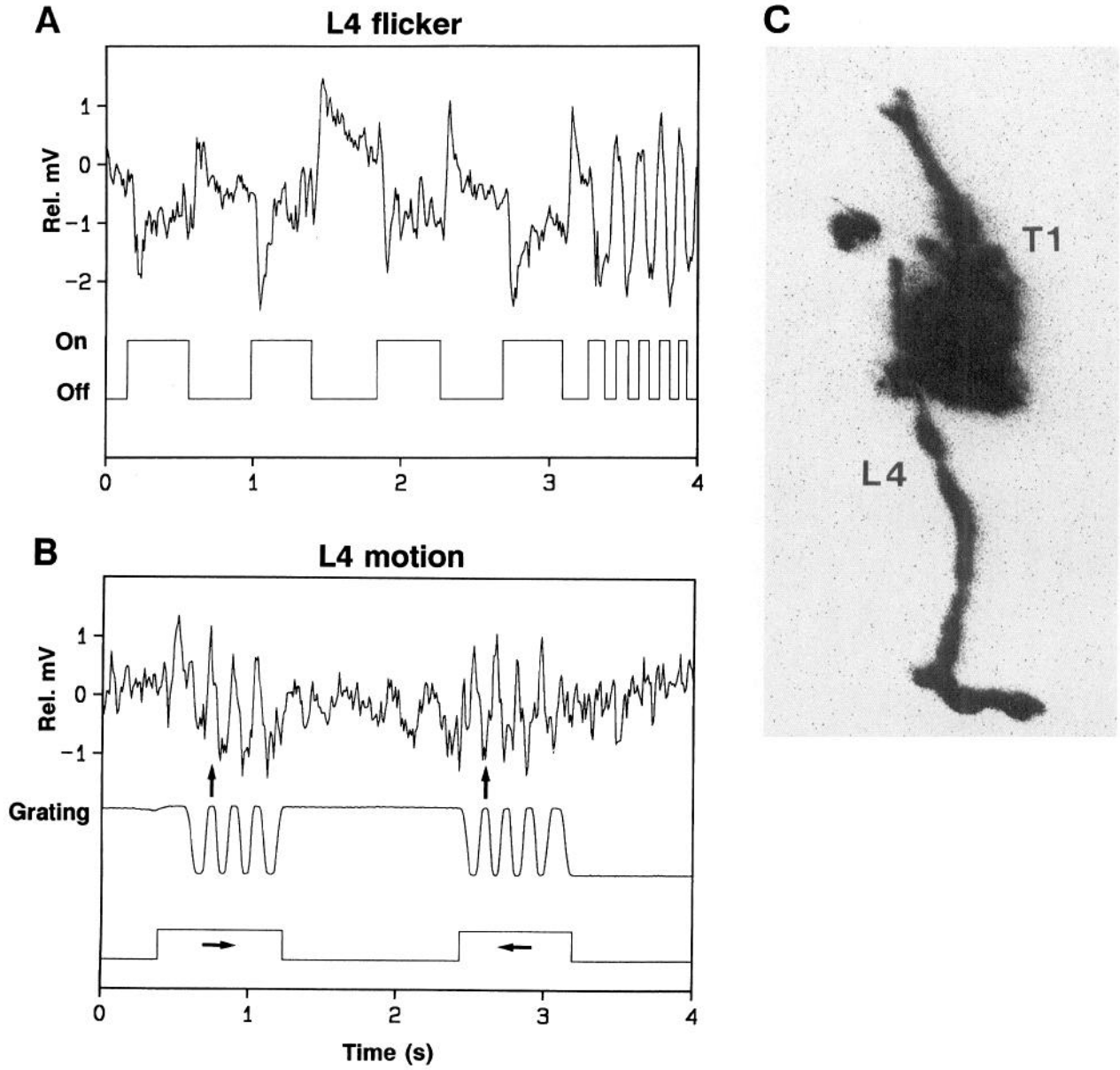


Figure 9. Responses by the L4 monopolar cell to flicker (*A*) show transient hyperpolarization to light-ON, and slowly decrementing depolarization to OFF. Depolarizations to motion (*B*) exhibit a direction-dependent phase shift (*vertical arrows*). See Discussion. *C,* Confocal projection (negative image) of the medulla terminal of L4 and the endings of a T1 stained en passant at a more superficial level. Magnification, 2000×.

mostly small, and project through relatively great distances, it was extremely difficult to capture the entire image because of bleaching. We subsequently used high-magnification confocal projections, which, even of apparently bleached out neurons, provided what must be the best resolution obtained of these cell types by any method. For example, the confocal image of T1's neurites in the L2 layer of the medulla (Fig. 8*C*) reveals far more of its morphological details than had been achieved in previous views of this cell (Järvilehto and Zettler, 1973; Strausfeld, 1976). Likewise, stereo confocal projections of the T5 dendritic tree in the lobula (Fig. 11) clarify the existence of both varicosities and fine spine-like processes that are obscured in Golgi-stained material, and which could otherwise only be painstakingly reconstructed from electron micrographs. The resolution of outswellings as well as spines suggests that T5 dendrites possess both presynaptic and postsynaptic sites within the dendritic tree, features that may be crucial in using physiological and structural data for modeling this system. In summary, then, each of the filled cells not only unambiguously corresponds to a cell type known to be distributed across the retinotopic mosaic, but provides even greater anatomical detail.

Cellular correlates to hypothetical elementary motion detectors

Do identified small-field neurons correspond to elements of the two most commonly proposed models of elementary motion detectors: the correlation and gradient models (for reviews, see Hildreth and Koch, 1987; Borst and Egelhaaf, 1989; Franceschini et al., 1989)? A generic correlation-type elementary motion detector is illustrated in Figure 12. Within the array of retinotopic sampling points, receptor elements (R) respond to local changes in light intensity. These changes may be the consequence of flicker, or of movement of a contrasting edge across

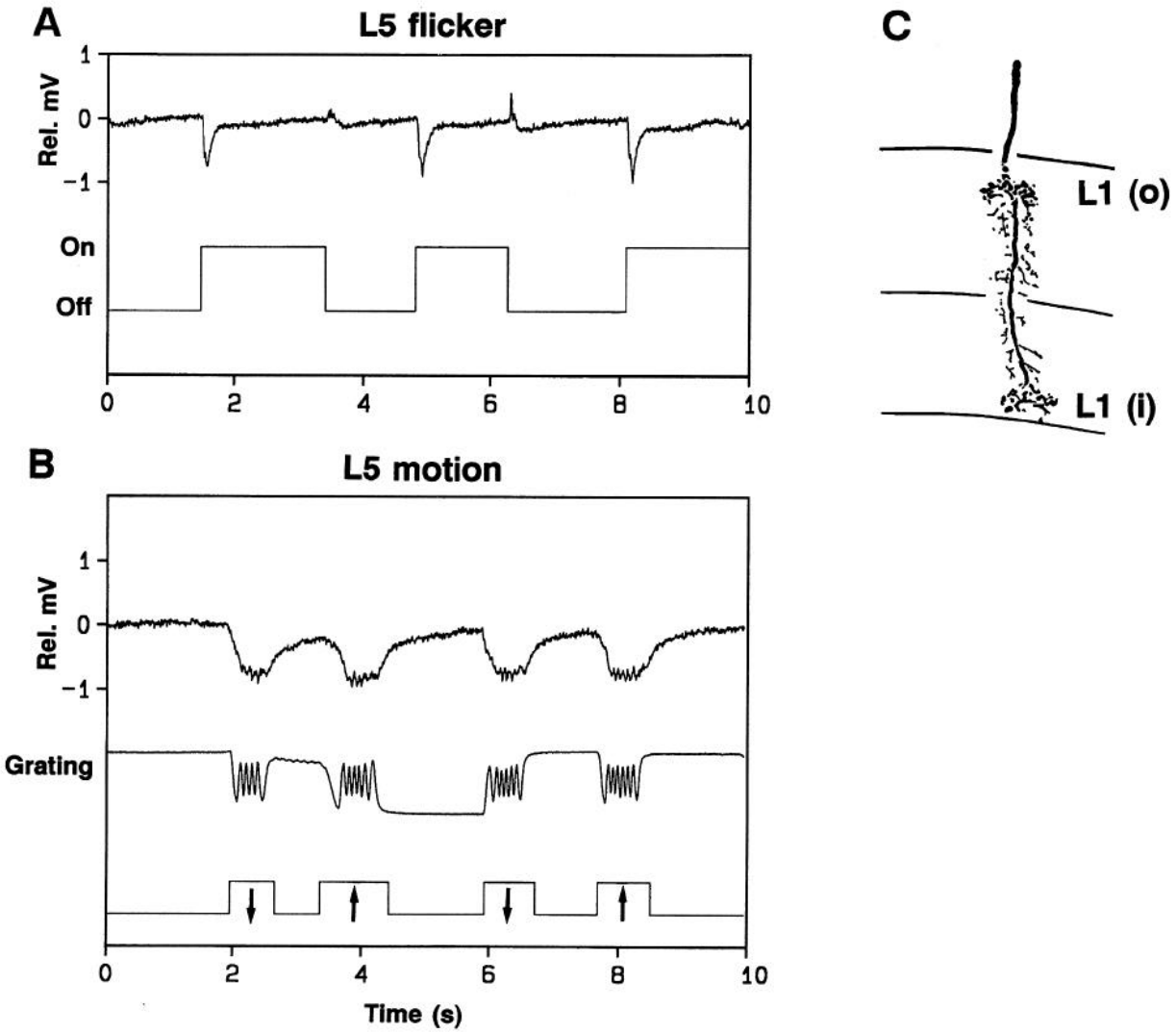


Figure 10. Responses to flicker (*A*) by the L5 monopolar cell are a transient hyperpolarization to light-ON with rapid recovery and a smaller amplitude transient depolarization to light-OFF. Response to motion in any direction (*B*) is a weakly phase-modulated sustained hyperpolarization. *C*, Reconstruction of the medulla terminals of this cell in the medulla, with outer (*o*) and inner (*i*) L1 layers indicated. Magnification, 625×.

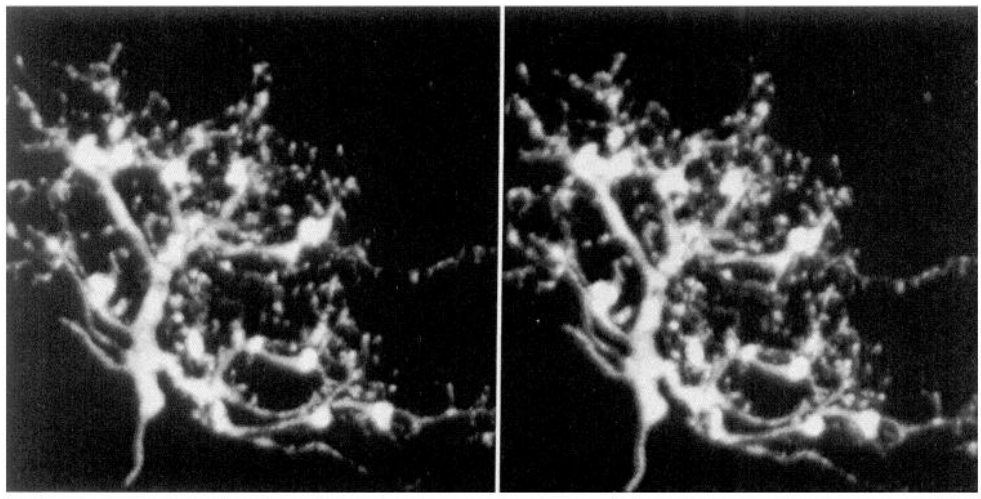


Figure 11. Stereo confocal projection of a T5 neuron dendritic tree. Note the coexistence on the same branches of varicosities and spines. Magnification, 1800×.

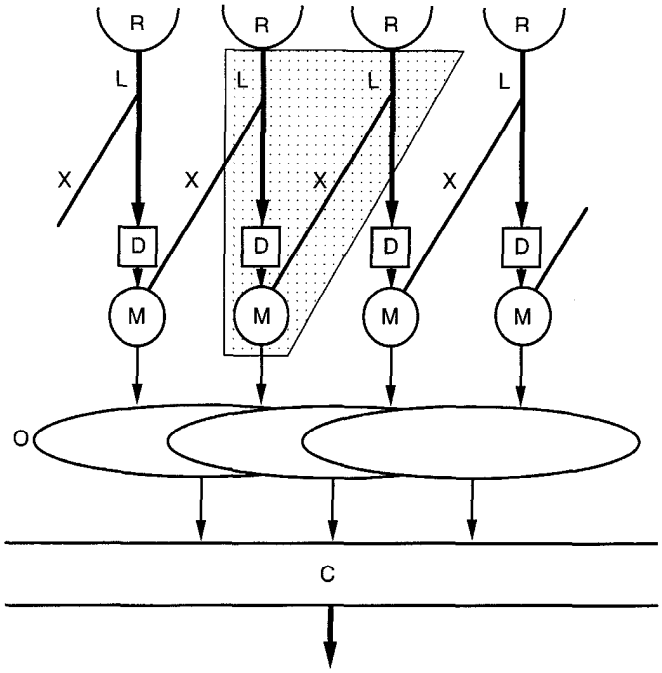


Figure 12. Generic circuit diagram for directionally selective correlation-type elementary motion detectors. The *stippled area* encloses a single emd. *R*, receptor; *L*, first-order relay; *X*, connector between neighboring channels; *D*, delay filter or long-pass filter; *M*, integrator; *O*, second integrating stage that combines outputs of several emds; *C*, collator that collects from assemblies of second integrators. Cell types possibly relating to elements of hypothetical emd are R1–R6 (*R*); LMCs (*L*); L4, C2 (*X*); SMCs, T1 (*D*); Tm1 (*M*); T5 (*O*); VS, HS, FD (*C*). See Discussion.

a receptor's receptive field. In order for motion to be detected, the responses of two receptors must be combined at an integrating stage (M) so that simultaneous activation (flicker) and sequential activation (motion) produce different outputs from the integrator. In correlation models, this is accomplished by delaying (or low-pass filtering) signal transmission in one channel (D) relative to the other channel (X). Sequential change of luminance in one direction thus results in the coincident arrival of responses at the integrating stage, while responses to change in the opposite direction reach M at different times. This generic correlation-type emd is thus directionally selective, since outputs in response to opposite directions of motion are different. Directional selectivity may then be further enhanced by combining (O) the outputs of two emds with opposite directional selectivities. Which direction of motion actually results in an excitatory output depends on whether the delayed channel itself has an excitatory (Hassenstein and Reichardt, 1956) or inhibitory (Barlow and Levick, 1965) influence on the second channel. Gradient models (Hildreth and Koch, 1987) share some of the basic characteristics of correlation models, but instead of delay elements, they employ a combination of edge detectors and flicker detectors.

Cellular correlates to emd steps

The first requirement for motion detection is for local intensity change detection. The next stage requires lateral interactions between channels representing neighboring visual sampling units, and asymmetrical delays among adjacent retinotopic columns. The obvious (and apparently ubiquitous) collateral organization of L4 originally suggested its role in motion detection (Braitenburg, 1970), or in lateral inhibition (Strausfeld and Campos-Ortega, 1977), a suggestion that has been vigorously contested by Shaw (1984). L4's responses to motion are not inconsistent with a role in motion detection, however. The observed depolarizations to grating motion could provide a delayed input to L2 relative to the direct inputs from R1–R6. The direction-dependent phase shift in L4's responses even suggests that directional information has already been computed at this level, although an alternative explanation, which future recordings will clarify, is a corresponding phase shift between the cell's receptive field and the center of the projection screen monitored by the light meter.

Another lamina monopolar cell which does not receive direct inputs from photoreceptor terminals is the midget monopolar cell L5 which appears to participate early in the initial stages of motion computation. L5 demonstrates a clear difference between its phasic responses to flicker and tonic responses to motion. Thus, L5 is in some sense motion sensitive, although there is no evidence for directional selectivity. L5 receives only a few synapses from wide-field elements in the lamina (which may protect it from wide-field stimulation) and its activity may reflect that of deeper retinotopic neurons in the medulla where its morphological relationships are intriguing. Golgi electron microscopy of L5 and of T1 (Strausfeld, unpublished observations) suggests that their terminal processes interpose between the LMC endings (L1 and L2) and small-field postsynaptic neurons such as iTm and Tm1. Both L5 and T1 seem ideally placed to play the role of a delay unit (D in Fig. 12), functioning as a local element between afferents from the lamina and subsequent retinotopic elements.

Where is the first level of directional sensitivity?

We have shown that the T5 cells from the outer lobula stratum respond to specific directions and orientations of motion. T4 neurons, whose dendrites are morphologically identical to those of T5, arise from the inner medulla stratum and are visited by the terminals of iTm. Recordings of motion-sensitive units (DeVoe, 1980) at, and motion-dependent 3H-2-deoxyglucose uptake by, the T4 stratum (Buchner et al., 1984) provide some evidence that T4 might be directionally selective. Golgi studies demonstrate converging inputs from Tm1 and iTm upon T5 and T4, respectively (Strausfeld and Lee, 1991). However, T5 (and possibly T4) are not the first neurons in the magnocellular pathway to show directionally selective responses. Tm1 is directionally selective, implying the existence of lateral interactions and delays either at or presynaptic to its dendrites in the medulla where L1 and L2 terminals are appropriately located to provide its main inputs, possibly via L5 and T1. It seems significant that these relationships are conserved among the Diptera (Buschbeck and Strausfeld, 1994; Buschbeck, unpublished observations), as is the organization of C2 neurons. C2's two levels of presynaptic specialization, one at the terminal of L1 and L2 endings, and a second above the L1, L2 dendrites in the lamina, suggest that this centrifugal cell type may play a major role in computing motion between neighboring channels. Because only C2's lamina specializations are GABAergic (Datum et al., 1986; Meyer et al., 1986), we suggest that C2, like the starburst amacrine cell in the rabbit (Masland and Tauchi, 1986), provides two species of synapses at its two levels of varicosities: an inhibitory GABAergic synapse onto L1 and L2 in the distal lamina, and a

second excitatory synapse, possibly cholinergic, onto L1 and L2 terminals in a neighboring column in the medulla.

T5 fits easily into the elementary motion detector pathway as a cell that is almost certainly postsynaptic to Tm1, since Tm1 terminals and T5 dendrites are the only elements sharing the outer stratum of the lobula, and responses of T5 and Tm1 accord with their assumed synaptic contiguity. T5 dendritic recordings, as well as axonal records, show this element to be both excited and inhibited by its inputs. The observed direction-dependent changes in the frequency of membrane fluctuations in Tm1, though seemingly more subtle than the positive and negative DC shifts in T5 and the lobula plate tangentials, are nevertheless sufficient in principle to alternately produce excitatory or inhibitory potentials in a postsynaptic cell equipped with appropriate receptors. A precedent for this phenomenon exists in the abdominal ganglion of *Aplysia,* where different kinetic properties of two acetylcholine receptors in a single follower cell have precisely this effect (Wachtel and Kandel, 1967, 1971). The converging Tm1 inputs onto T5 may function to amplify rather than generate directional information. If so, a portion of the amplification could be accomplished by appropriately integrated inputs from Tm1s with different directional selectivities. The role of T5, in turn, would be to transform and further amplify the signal before its delivery to the lobula plate. Thus, with regard to the emd model, it would be Tm1 that corresponds to the integrator (M, Fig. 12), with T5 being a small-field summatory unit. The giant lobula plate neurons (e.g., VS) to which T5s are presynaptic (Strausfeld and Lee, 1991) would thus serve to collate information about directional motion from large arrays of T5 neurons.

Appealing as the above scenario may be, a number of details regarding synaptic connections, neurotransmitters, and their receptors remain to be investigated. What is clear from the T5 recordings, and what has been absent from previous models of the fly visual system, is that these small-field inputs to the HS and VS system are fully directionally selective, with both excitatory and inhibitory responses to motion. Previous investigations comprising electrophysiological recordings from tangential cells (Gilbert, 1990; Egelhaaf et al., 1993), theoretical investigations (Borst and Egelhaaf, 1989; Egelhaaf and Borst, 1992), and pharmacological studies (Warzecha et al., 1993) led to the widespread expectation that individual neurons presynaptic to HS and VS cells exhibit a unidirectional response to motion, with the final integration of these responses taking place at or near the dendrites of lobula plate tangential cells. In light of the present findings, this assumption should be revised: unidirectional responses now appear to arise at least two synapses prior to the wide-field tangential cells.

Parallel small-field channels to the lobula plate

The nature of T4 cells remains an intriguing puzzle of elementary motion detection. Their striking morphological similarities to T5 (Strausfeld and Lee, 1991; Buschbeck and Strausfeld, 1994), the similarity between T5 and T4 cells' presumptive small-field inputs (respectively Tm1 and iTm), and their similar synaptic connections onto lobula plate VS and HS tangential giants (Strausfeld and Lee, 1991) all indicate that T5 and T4 are physiologically similar. On the other hand, although its response to motion is distinct from its response to flicker, iTm, the putative input to T4, differs from the Tm1 input to T5 in that it so far shows no evidence for directional selectivity. However, the mere presence of two parallel pathways to the lobula plate (see Fig. 1) clearly suggests some degree of computational specialization. Anatomical studies show that each visual sampling point is represented by four T5 and four T4 cells (Strausfeld and Lee, 1991), all of which segregate to the HS and the VS layers in the lobula plate. Thus, because T5 responses are directionally hyperpolarizing or depolarizing, all the necessary inputs to the lobula plate optomotor tangentials could be provided by the T5 pathway alone, without invoking the participation of T4 neurons.

Early motion computation for optokinetic responses is ubiquitous

In conclusion, the present results clearly show directional responses to motion in the transmedullary cell Tm1 and the bushy T-cell T5. The results also provide evidence for early motion sensitivity in cells that cross the chiasma between the medulla and the lamina (C2, L5, and possibly L4). The idea that motion detection takes place very early in visual processing is well established for vertebrates (Maturana and Frank, 1963; Werblin et al., 1988; Yang and Masland, 1993) but similar evidence has, until now, been lacking for arthropods, despite the clear importance of rapid optomotor reflexes. The one possible exception is the water flea *Daphnia,* where there are only two visual neuropils (lamina and medulla; Consi et al., 1987) and where there is some evidence that motion and light-ON can elicit eye flicks mediated as few as two synapses distant from the photoreceptors (Consi et al., 1985, 1990). The present results suggest that elementary motion detection in insects also begins at an early level of visual processing.

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