Retinotopic Pathways Providing Motion-Selective Information to the Lobula From Peripheral Elementary Motion-Detecting Circuits

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ABSTRACT

Recordings from afferent channels from the medulla supplying deep neuropils of the fly’s optic lobes reveal different filter properties among the three classes of afferent neurons: transmedullary cells, T2 neurons, and Y cells. Whereas transmedullary cells respond to local flicker stimuli without discriminating these from directional or oriented motion, the T2 afferent neurons show clear motion orientation selectivity, which corresponds closely with a morphological bias in the orientation of their dendrites and could also be influenced by systems of local recurrent neurons in the medulla. A Y cell having a clearly defined terminal in the lobula, but having dendrite-like processes in the medulla and, possibly, the lobula plate, discriminates the direction of motion and its orientation. These results demonstrate unambiguously that the lobula receives information about motion and that the channels carrying it are distinct from those supplying wide-field motion-selective neurons in the lobula plate. Furthermore, recordings from a newly identified recurrent neuron linking the lobula back to the inner medulla demonstrate that the lobula discriminates nondirectional edge motion from flicker, thereby reflecting a property of this neuropil that is comparable with that of primary visual cortex in cats. The present findings support the proposal that elementary motion detecting circuits supply several parallel channels through the medulla, which segregate to, but are not shared by, the lobula and the lobula plate. The results are discussed in the context of other intracellular recordings from retinotopic neurons and with analogous findings from mammalian visual systems. J. Comp. Neurol. 457:326–344, 2003.

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Neuroanatomy and intracellular recordings have elucidated many features of neural circuitry underlying visual motion detection and processing by small-field retinotopic neurons in flies (Douglass and Strausfeld, 2001, and in press). Achromatic optomotor pathways begin with inputs from the R1–6 photoreceptors to the pair of large lamina monopolar cells, L1 and L2, and various other lamina output neurons (Boschek, 1971). These afferent neurons terminate in the medulla, where they supply a system of retinotopic relays that supplies arrangements of directional motion-sensitive neurons that eventually synapse onto wide-field tangential neurons of the lobula plate (Fig. 1A,C). Crucial players in this organization are the Tm1 small-field transmedullary cells that terminate at the dendrites of T5 direction-sensitive cells that extend from a superficial layer (the T5 layer) of a second deep visual neuropil, called the lobula (Fig. 1A). These retinotopically organized neurons together provide information about op-
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...tic flow patterns generated by self-motion (Strausfeld and Lee, 1991; Douglass and Strausfeld, 1995, 2000a,b; Krapp et al., 1998). Lobula plate tangential neurons, in turn, provide signals that are relayed to descending neurons that contribute to the control of direct flight muscle motor neurons and neck muscle motor neurons (Strausfeld, 1997).

The lobula is supplied from the medulla by a very large number of different morphological types of retinotopic neurons (Fig. 1B,C). About half of these cell types supply the lobula exclusively; the other half, called Y cells, send bifurcating axons to the lobula and the lobula plate (Fig. 1C). Although the role of the lobula in motion processing is still obscure, evidence from various studies demonstrates that it receives afferents that allow directional and non-directional motion signals to be used to generate visually guided behaviors. For example, lesioning the axons of HS and VS cells and other efferents of the lobula plate demonstrates that optomotor control can still be accomplished and, therefore, does not depend solely on outputs from that optic neuropil (Strausfeld et al., 1995). In addition, several species of large-diameter neurons found only in the male lobula (Strausfeld, 1991) show direction-selective properties (Gilbert and Strausfeld, 1991) and fulfill the requirements for a system of neurons that supports target tracking, pursuit, and interception (Collett and Land, 1978; Gronenberg and Strausfeld, 1991). In dragonflies, intracellular recordings that were attributed to the odo-nate lobula demonstrate sensitivity to orientations, rather than directions, of moving-bar stimuli (O’Carroll, 1993).

A fourth system of retinotopic neurons comprises feedback neurons (Fig. 1D). These link deep levels of the medulla to more peripheral levels and connect the medulla centrifugally to the lamina. A new class of feedback neurons is described here linking the lobula back to the medulla.

Findings from the fly raise an important question about the nature of motion-detecting pathways. Does a single set of “elementary motion-detecting (emd) circuits” provide inputs to several parallel pathways that diverge to the lobula plate and lobula? Alternatively, might discrete motion-detecting circuits separately supply these two neuropils? These questions concern a basic problem in vision research: Is motion detected at multiple processing levels, or does a single peripheral array of elementary motion-detecting circuits supply all higher visual centers? For dipterous insects, anatomical studies suggest that, already at the level of lamina monopolar cells, retinotopic pathways begin to diverge to several parallel channels (Strausfeld and Lee, 1988; Bensenwein and Fischbach, 1992). The presence of motion-sensitive inputs directly from the medulla to the lobula suggests that emds might reside peripherally in the system and that these might supply divergent channels to a variety of deeper targets.

One approach to answering these questions is to study the properties of neurons that link the medulla with the lobula and lobula plate and determine the levels at which such neurons originate in the medulla. Afferent pathways for delivering small-field, retinotopically organized, and motion-specific information from the medulla to the lobula can be categorized according to the relationships of identified cell types to the lobula. Transmedullary (Tm) cells arise from cell bodies lying distal to the medulla. They provide relays from inputs from the retina and lamina. Tm cells are arranged as retinotopic palisades, usually with one centered at each column, the column representing one visual-sampling unit (Franceschini et al., 1989) of the retina. Approximately 40 morphological species of Tm cells have been recognized (Strausfeld, 1970, 1976); the terminals of which reach restricted and characteristic levels in the lobula (Strausfeld, 1976). One family of Tm cells projects only to the superficial T5 layer covering the lobula, where it visits the dendrites of “T5” cells, arranged as quartets for every visual-sampling point of the retina (Strausfeld and Lee, 1991). T5 cells, which originate from cell bodies lying behind the lobula plate, supply four discrete levels within this neuropil. Tm cells supplying the T5 layer have no direct interaction with the deep lobula. These deep levels are supplied by members of a second family of Tm cells, most of which have dendritic fields extending across several retinotopic columns in the medulla. The terminals of these neurons end at characteristic levels in the lobula, where they coincide with ensembles of pyramidal-cell-like dendrites belonging to projection neurons that send axons to discrete regions of the lateral protocerebrum. In addition, the medulla also supplies the lobula with a second class of neurons, called T2 and T3 cells (Strausfeld, 1970), which originate from cell bodies beneath the medulla. These neurons send axons through the lobula’s T5 layer, to terminate among dendritic pali-sades of lobula projection neurons (Fig. 1B,C). A third class of medulla efferents (Fig. 1C) comprises the Y cells, so named because their axons bifurcate to link corresponding retinotopic loci in the medulla, lobula plate, and lobula. One class of Y cell (called transmedullary Y cells) is derived from cell bodies distal to the medulla. Another class arises from cell bodies beneath the medulla.

The present account describes for the first time clear evidence for orientation-selective as well as direction-selective responses by afferent neurons that originate in the medulla and supply the lobula. Neural organization in the medulla suggests that specific layers of this region might be involved in orientation-selective motion processing. The projection of these cells in the lobula indicates that such neurons supply pathways that are independent of the lobula plate in providing motion-specific information to the brain. The identification of a feedback pathway from the lobula to the medulla, which discriminates between edge motion and flicker, provides further evidence that the lobula is supplied with afferents that carry motion information. Until now, there has been no physiological evidence for direct inputs to the lobula that would account for the direction- and orientation-selective properties of lobula neurons. The present observations lend support to the notion of a peripheral emd that supplies divergent channels that separately supply the lobula and lobula plate with information about motion. This account also provides new insights into the manner in which motion-specific information may be parsed out among pathways that specialize in processing distinct aspects of motion inputs. We show that T2 cells deliver orientation-specific information directly from the medulla to the lobula, and present evidence that morphology at the T2 cell dendrites might help to explain selectivity for motion orientation. In contrast, we show that certain retinotopically organized Tm neurons that project to deep strata of the lobula are not specifically tuned to motion, but that a neuron with dendrites at levels that overlap with the endings of these Tm neurons reciprocally supplies edge-motion feedback to the
inner layer of the medulla (Fig. 1D). The discovery of this pathway suggests that parallel channels concerned with motion processing may be modulated by reafferent feedback.

MATERIALS AND METHODS

All experiments employed the calliphorid fly Phaenicia sericata. These were bred and are maintained as a laboratory colony at the Arizona Research Laboratories Division of Neurobiology. Eggs from locally trapped individuals were collected several times per year to help maintain genetic fitness. The basic methods for fly rearing, computerized visual-stimulus presentation, intracellular recordings, and histological processing were similar to those described in previous publications (Douglass and Strausfeld, 1996, 1998). Modifications of earlier procedures and instrumentation are summarized below. With the exception of the lobula-medulla feedback cell, all preparations were from males.

Stimuli and electrophysiology

Adult flies were immobilized with a low-melting-point mixture of beeswax and violin rosin and fixed to a pedestal such that the fly’s head was positioned 18 cm from the center of a computer monitor that was used to present visual stimuli. The pedestal was attached to a vibration-damped isolation table (TMC 78 Series; Peabody, MA) under a Faraday cage, with the fly’s head aligned for a frontal view of the monitor. The head was tilted downward at 45° from the horizontal position, providing access to the optic lobes from a hole cut in the cuticle at the back of the head. In most experiments, the thorax and abdomen were fixed at 45° from horizontal, bringing the head of the fly to a horizontal position. Single neurons were impaled within the medulla, the second optic chiasma, or the lobula complex by using sharp borosilicate or quartz pipettes fabricated with a laser puller (P2000; Sutter Instruments, Novato CA) and backfilled with Lucifer yellow CH (lithium salt; Molecular Probes, Eugene, OR) and 0.1–0.2 M LiCl. The pipettes, with typical in situ resistances from 80 to 270 MΩ, were connected to the head stage of an intracellular amplifier (usually an Axoprobe 1A or AxoClamp 2B; Axon Instruments, Foster City CA), and the head stage was attached to a micromanipulator (Leica). In some experiments, the manual fine advance of the micromanipulator was augmented by a piezoelectric drive (IW-800; Burleigh Instruments, Fishers, NY) that was mounted on the manipulator and configured to advance the pipette in rapid, 0.5-μm steps. Both the amplifier “buzz” and the piezoelectric drive were used to assist in penetrations.

During intracellular recordings, visual stimuli were presented using a custom monochrome monitor equipped with ultrashort-persistence green “P46” phosphors and operated at a horizontal refresh rate of 200 Hz (Vision Research Graphics, Durham, NH). At the fly’s head, the computerized display was 80° wide by 62° high. Below the fly and in front of the CRT, a small front-surface mirror, lens, and diaphragm aperture were arranged to image the center of the CRT screen onto a photodiode (PIN 10DP; UDT Sensors, Hawthorne CA). The output of the photodiode, amplified using a current-to-voltage op amp circuit, was used to monitor the stimulus timing and relative intensities. The fly viewed the CRT monitor through a square window cut in the side of the Faraday cage. The preparation was shielded from high-frequency CRT-generated noise by an indium-titanium oxide-coated glass plate (Thin Film Devices, Inc., Anaheim, CA) placed across the window and grounded to the Faraday cage. The above-described methods apply to all of the recordings.
presented here, except for one case (see Fig. 2A,E–H), in which a 160-Hz-refresh-rate monitor was used (FlexScan FX C6; Nanao, Matto, Japan) and the fly was positioned at 11 cm from the CRT.

Visual stimuli were generated with Neurosequence software (version 3.0; Vision Research Graphics) and consisted primarily of full-screen square-wave flicker and directional motion stimuli (see Results for details of individual recordings). Wide-field flicker was presented as a frequency “ramp” stimulus, with the flicker frequency increasing from 1 to 100 Hz at intervals that were evenly spaced on a logarithmic scale, followed by an equivalent series of decreasing flicker frequencies. Constant-velocity wide-field grating motions (50°/second) were presented at eight motion directions and one or more spatial frequencies ranging from 0.05 to 0.125 cpd (20° to 8° per cycle). Directional grating motion could also be presented at sinusoidally varied velocities from 0° to 200°/second. In some experiments, grating motion was augmented by directional motion of a single bright or dark edge that was moved horizontally (left or right) and vertically (up or down) at 50°/second. Reflections across the space between the CRT screen and the glass window in the Faraday cage potentially can produce dim “ghost” images that are displaced from the positions of primary images. As a precaution against this, the difference between the brightest and dimmest stimulus intensities was chosen to render ghosts invisible to human observers.

Intracellular voltages were amplified and stored along with stimulus timing signals on VHS tape, by using a VCR equipped with a PCM recording adapter (Vetter 3000A; Vetter, Regensburg, PA). The raw data were subsequently replayed from the VCR and digitized by using a Power 1401 and Spike 2 software (version 4; Cambridge Electronic Design (CED), Cambridge, United Kingdom).

**Histology and anatomical reconstructions**

At the end of each experiment, the recorded cell was injected iontophoretically with Lucifer yellow (Molecular Probes) by using a steady, 1–3-nA, hyperpolarizing current for 1–3 minutes, and the brain was removed from the head capsule and fixed overnight in 4% formalin in Millonig’s buffer at pH 7.2. Fixed brains were rinsed twice in Millonig’s buffer, dehydrated in ethanol followed by acetone, embedded in a modified version of Spurr’s (1969) plastic, and polymerized at 70°C for 12 hours. Serial 14-μm horizontal or vertical sections were cut with a sliding microtome, mounted on slides, and coverslipped with fluoromount (Serva, Heidelberg, Germany). Profiles of stained cells were scanned at 0.3–1.0-μm intervals (depending on magnification) with confocal epifluorescence microscopes equipped with an argon laser (Nikon PC 2000 and Zeiss LSM 5 Pascal systems).

Anatomical relationships between intracellularly stained neurons and the surrounding neuropil architecture were revealed by obtaining confocal images at low magnification. Morphologies of filled neurons were then reconstructed from higher-magnification confocal projections of individual serial sections, or the confocal projections from separate plastic sections were merged using any of several image-processing software packages: Confocal Assistant 3.1 (Todd Brejle), Corel Photopaint 5 or 6 (Corel, Salinas, CA), Zeiss Pascal (Zeiss, Thornwood, NY), and Simple PCI (Compix, Cranberry Township, PA). Serial confocal images were then reconstructed as black-and-white profiles. In one preparation of a T2 cell, rotated three-dimensional (3-D) views of its dendritic arborizations in the medulla were constructed from high-magnification confocal stacks by making Simple PCI “movies.”

**Light microscopic methods**

Opened heads were treated with the combined Golgi Colonnier-Golgi rapid method described in other accounts (Strausfeld and Li, 1999). Reduced silver preparations were made from serial 10-μm Paraplast Plus sections using Bodian’s (1937) original method. Mass impregnation of medullary neurons was achieved by cobalt diffusion into the second optic chiasma, followed by sulfide precipitation and silver intensification, as described by Bacon and Strausfeld (1980).

**Neuron reconstructions**

Dendritic trees and terminals of Golgi-impregnated neurons were reconstructed from stacked optical sections captured with a Sony DRC 5000 CCD digital camera linked to an Apple G4 computer equipped with graphics software. Images were captured at an initial magnification of ×600, using a planapochromat oil-immersion objective. For each thick section, between 8 and 25 optical images were captured and layered in register. Sections were made transparent using the Adobe Photoshop darkening function. Shadows were removed, and the images were flattened. This procedure results in images of Golgi-impregnated neurons that show each process exactly in focus throughout a depth of up to 50 μm.

**Data analysis**

Spike 2 software (CED) and custom Spike 2 scripts were used to produce raw data plots and for some quantitative analyses (see Results for details). Some intracellular data were digitally filtered using Spike 2 or Origin 6.1 (OriginLab, Northampton, MA) prior to analysis in order to attenuate low-frequency baseline fluctuations or 60-Hz line noise. In other cases, the data were digitally low-pass filtered to illustrate lower frequency changes of interest. Spike counts and instantaneous spike frequencies were obtained by using in-house event-detection programs written in Pascal (Borland International).

Data were plotted by using Origin 6.1 (OriginLab). Basic processing of confocal images was performed with Adobe Photoshop 5.5 and/or Corel Photopaint 8.0. Three-dimensionally rotated views of confocal stacks were generated with Simple PCI and/or Zeiss Pascal software. All final figures were assembled in Adobe Photoshop 5.5 or Corel Draw 8.0. Directional data were evaluated by plotting on polar coordinates and calculating a mean (preferred) angle or mean (preferred) orientation as described by Batschelet (1981).

**RESULTS**

The medulla comprises two regions, called the outer and inner medulla, that are separated by the serpentine layer carrying axons of wide-field neurons from and to each of these layers. Both the outer and the inner medulla are composed of precisely arranged retinotopic columns that are transected at various levels by discrete tangential strata (Fig. 2D). The outer and inner medullas differ, however, in that afferents from the retina (R7 and R8
Figure 2
photoreceptor endings; Campos-Ortega and Strausfeld, 1972a) terminate only in the outer medulla. The inner medulla is characterized by its wide-field amacrine cells and recurrent afferent endings. Most of the latter are supplied from the midbrain, but some described below are derived from the lobula and from the lobula plate.

Over 40 types of efferent neurons link the medulla to the lobula and lobula plate and supply them with higher-order visual primitives, from which neurons in these neuropils reconstruct the visual world. It is likely that responses of medulla efferent neurons are shaped by systems of local interneurons. Some are axonal and serve to shunt information between different levels of the medulla. Others comprise the large variety of amacrine-like neurons that are situated at discrete strata. Whether efferent or local, each medulla neuron type is uniquely identifiable morphologically (Fig. 2C) and can be recognized repeatedly in different preparations. Many can be recognized across taxa (Buschbeck and Strausfeld, 1996).

**Direct retinotopic inputs to the lobula from the medulla: the Tm cells**

Tm cells make up the main class of neurons providing direct retinotopic inputs from the medulla to the lobula. There are approximately 40 uniquely identifiable Tm-cell architectures, of which only four belong to Tm cells that terminate in the outer T5 dendritic layer of the lobula (the neurons Tm1, Tm1a, Tm1b, Tm9; Douglas and Strausfeld, 1995, 1998). Others send their axons directly through this layer and terminate at deeper levels of the lobula. These deep Tm cells include the type 2 Tm cell (Tm2), which has been recorded from previously and shows no evidence of selectivity for motion (Douglas and Strausfeld, 1998). Here we present recordings from two additional Tm-cell types that end deep in the lobula among columnar projection neurons that supply regions of the lateral protocerebrum.

Confocal reconstruction (Fig. 2A) and physiological recordings (Fig. 2E–H) have been obtained for a small-field Tm cell that has diffuse arrangements of dendrites in the outer medulla, spanning some five to eight retinotopic columns at the level of L1 monopolar cell endings and the endings of R7 and R8 photoreceptor axons from the retina. It also provides lateral processes within the inner medulla. Its responses to flicker (Fig. 2E,F) and directional grating motion (Fig. 2G,H) consisted mainly of hyperpolarizations in phase with the stimulus. There was no evidence for direction- or orientation-specific responses.

A second Tm cell has been identified, the dendrites of which in the medulla are restricted to levels immediately above and below the serpentine layer (Fig. 2B). Of special interest is that this cell type provides a tight cluster of short dendrites within its parent retinotopic column and longer and more sparsely distributed dendrites extending obliquely vertically away from the column to transect others. This morphological arrangement suggests a central area of input for this neuron, at the terminal level of L1 monopolar cells, with lateral processes that may provide a morphological basis for center-surround organization, although we were not able to test this physiologically. Its processes in the inner medulla are in a position to receive inputs from the lobula-to-medulla feedback neuron described below as well as from a variety of other afferent endings within this layer. The axon of this neuron extends about halfway through the depth of the lobula, reaching the level of deep columnar output neurons.

This Tm cell responded to wide-field flicker (Fig. 3A,B) with transient On-hyperpolarizations and Off-depolarizations that are similar to those often observed in other small-field peripheral neurons in the optic lobes of flies, with the exception that the present responses lacked a sustained hyperpolarization. Responses to grating motion with sinusoidally varied speeds (Fig. 3C,D) were characterized by small, contrast-frequency-dependent voltage fluctuations that provide no evidence of selectivity for motion direction or orientation. As is discussed below, these results are consistent with previous recordings suggesting that Tm cells might not be involved with conveying directional motion information to the lobula.

**Orientation-specific inputs from the medulla to the lobula provided by type 2 T cells**

T cells are retinotopic neurons that provide direct input from the medulla to the lobula plate, via T4 cells, or to the lobula, via T2 and T3 cells. T3 and T4 neurons are distinguished by dendrites restricted to the inner medulla (Strausfeld, 1970).

The T2 cells are unusual in that their main trunks extend to almost the surface of the outer medulla, providing sometimes two but usually three levels of dendrite-like arborizations (Fig. 4A). T2 cells send their axons to midway through the lobula, where they end as stout, varicose terminals (Fig. 4C). The outermost dendrites of T2 reside at the same level as the outer swellings of L1 monopolar cells above the terminals of the L2 monopolar cell (Fig. 4C,E). A layer of dendrites immediately beneath lies at the level of T1 endings from the lamina (Fig. 4C). This basic morphology of T2 has been described from Golgi preparations from *Musca domestica* (Strausfeld, 1970, 1976; Campos-Ortega and Strausfeld, 1972b) and from *Drosophila* (Fischbach and Dittrich, 1989), suggesting that it may be widespread among Diptera. As with the T3 monopolar cell, T2 neurons originate from a cluster of cells situated beneath the posterior inner face of the inner.
Fig. 3. Intracellular responses of the transmedullary cell illustrated in Figure 2B. A: Transient On-hyperpolarizations and Off-depolarizations during wide-field flicker-ramp stimulus. B: Expanded view from A, showing responses to fast flicker. C, D: Responses to directional grating motion, the speed and directions of which (lower panels) were varied sinusoidally between 0 and 200°/second.

The T2 cell reconstructed in Figure 5 was unambiguously identified with a physiological recording of unusual stability, possibly accounted for by the stout (3–5-μm-diameter) central trunk and axon of this cell type. This allowed tests not only with flicker and directional-motion stimuli but also with directional motion at three spatial frequencies ranging from 0.13 to 0.05 cycles per degree (cpd; 8° to 20°/cycle).

The responses of T2 to flicker (Fig. 5B,C) were strong only to fairly low temporal frequencies (less than approximately 5 Hz) and consisted of transient On-hyperpolarizations and Off-depolarizations similar to those of the Tm cell shown in Figure 2. Notably absent was the sustained On-hyperpolarization that is usually observable in recordings from Tm cells, LMCs, and other peripheral visual interneurons.

T2 responded to grating motion (Fig. 5D–F) with spike-like depolarizations that varied in frequency and amplitude, and occasionally also exhibited slow depolarizations unaccompanied by prominent spiking. No significant hyperpolarizations were observed in response to motion. Responses to grating motion depended on motion direction, and showed increased frequencies of depolarizations at the higher contrast frequencies associated with higher spatial frequencies.

Quantitative comparisons of responses to different motion directions were made by subtracting mean prestimulus baseline voltages (integrated from the 250 msec period just prior to the start of motion) from the mean voltage during motion to yield a measure of response intensity. The results were plotted using polar coordinates. As illustrated in Figure 6A–C, the overall patterns of responses at three spatial frequencies show selectivity for the orientation of motion. Orientation selectivity is clearest for the 0.13-cpd grating. The mean response to all three spatial frequencies (Fig. 6D) clearly shows a preference for oblique, upward-progressive and downward-regressive motion directions, with a bimodal distribution of response amplitudes and a mean axis (preferred orientation) of 161°. In Figure 6, mean responses for each spatial frequency are shown normalized. Overall means and standard errors given in Figure 6D were calculated from the raw data; when calculated from normalized data, the results were virtually identical.

Possible morphological substrates for the orientation selectivity of T2

Cross-sectional views of T2 along the full length of its projections in the medulla were made by rotating confocal stacks around an axis perpendicular to the longitudinal axis of T2’s columnar trunk (Fig. 7A). The rotated views (Fig. 7B) reveal the arrangement of T2 branches in tangential planes. These were matched to the retinotopic map at corresponding levels. The radial distribution of T2 arborizations was measured from each rotated image by superimposing it on a radial grid divided into 22.5° sectors (Fig. 7D) and counting the number of grid regions in which a portion of the T2 dendritic field was visible within each sector. Most of the distributions of dendritic orientations with respect to the axes of the retinotopic mosaic (Fig. 7C) showed a strong tendency toward a shared, oblique (diagonal, with respect the horizontal axis of the eye), preferred orientation. Summing the individual distributions (Fig. 7E) not only confirms but amplifies this tendency, showing a preferred orientation with a mean axis of 139°. The most interesting aspect of the morphological orientation, however, is its similarity to the preferred axis of motion orientation that was measured from physiological responses to motion.

These morphological orientations correspond to the mainly oblique axes of the retinotopic mosaic. Golgi impregnations of T2 dendrites (Fig. 4D) at the outer level of the medulla show its extended orientation along the “X” and “Y” axes of the retinotopic mosaic. However, such orientations do not necessarily imply physiological-orientation selectivity. Commonly associated with T2 neurons is a novel class of columnar intrinsic neuron, the
Fig. 4. T2 neurons (A–E) and their associated intrinsic medullary centrifugal neuron (I–K). C: Reconstruction of an intracellularly filled T2 cell, rotated through about 45° to show its vertical aspect. The perpendicular trunks of T2 cells extend outward from the inner margin of the inner medulla to the outer surface of the outer medulla, where a small cluster of dendrites coincides with the outer swelling of the bistratified L1 monopolar cell terminal (D). These dendrites have a characteristic lateral extension (arrows in D,E) disposed distally to the L2 monopolar cell terminal (E) and oriented obliquely with respect to the medulla’s vertical axis (G,H). T2 collaterals in the inner medulla reside immediately distal to the layer of T4 dendrites (bracketed in A–D,J,K) and coincide with the deep dendrites of medulla intrinsic centrifugal cells (J,K). A second variant of the T2 cell lacks these collaterals (cell at right in B). T2 dendrites in the middle of the outer medulla (at level of boxed areas in A,C,D) coincide with the midmedulla collaterals of centrifugal cells (boxed area in J). Centrifugal cells originate from cell bodies lying immediately above the outer medulla, each providing a neurite that fuses with the perpendicular trunk of the neuron, just distal to the serpentine layer. Centrifugal cells provide beaded processes (arrows in F,J,K) that branch among T2 dendrites. Processes also occur at the levels of L2 and T1 terminals (J,K). Viewed normal to the medulla’s depth (I), these terminals provide a plexus across the medulla’s outer stratum. Scale bars = 25 μm.
dendrites of which are disposed to provide recurrent feedback from the level of T2's deeper dendrites back out to its most distal branches. These neurons, here called medulla intrinsic feedback neurons, which are shown in Figure 4F,J,K, provide clusters of bleb-like terminals at the level of the terminals of T1 and L2 neurons from the lamina and the outer swellings of the L1 monopolar cell. The axons of these feedback neurons entwine with the columnar trunk of T1, and the terminals of the feedback neuron interweave among the outer dendrites of T2 (Fig. 4J,K). Top-down views of these medulla feedback neurons (Fig. 4I) show that their terminal domains spread across several columns and overlap. Possibly this arrangement provides alternative pathways for establishing T2 orientation selectivity, employing systems of lateral inhibition among linear arrays of T2 cells that might provide inputs to feedback neurons at levels of the inner medulla.

Y cells and direction-specific inputs to lobula

Y cells connect the medulla, lobula plate, and lobula. There are two basic varieties of Y cells; transmedullary Y (TmY) cells that arise from cell bodies distal to the medulla and include some 15 identified retinotopic types (Strausfeld, 1970, 1976) and Y cells that arise from cell bodies proximal to the medulla. Recordings to date (Gilbert et al., 1991; Douglass and Strausfeld, 1998) suggest that TmY cells distinguish motion from flicker but are not concerned with directional-motion processing. Among the second class of Y cells, five types have been identified, two from Golgi preparations (Strausfeld, 1976) and three from intracellular penetrations and dye fills (Douglass and Strausfeld, 1996, 1998). Here, we describe physiological responses and new anatomical details of the neuron Y19,
possesses motion-specific responses to both single-edge and grating motion. The responses to grating motion were also unusual, and differed from the Tm cell responses described above, in that there were two cycles of On/Off-like depolarization for the passage of each stripe.

Analysis of the motion responses (Fig. 8H) clearly shows that this cell is directionally selective. As is typical of many direction-selective neurons, the directional tuning is broad. Individual responses to moving gratings were strong to both upward and horizontal motion, resulting in a mean preference for upward motion (Fig. 8H, arrow). The dendrite-like nature of Y19’s arborizations in the medulla and lobula plate suggests that the directional sensitivity of this cell could arise from inputs either to the medulla or to the lobula (see Discussion).

Feedback from the lobula to the medulla

Many visual interneurons have obvious polarities with respect to the fine structure of their branches. For example, the intrinsic medullary centrifugal neuron (Fig. 4J) is clearly equipped with beaded terminals at one level and slender tapering dendrites at other levels. Certain identified neurons appear, based on similar criteria, to provide feedback from deeper to more peripheral processing levels. Little is known about the functional roles of these neurons, except for the C2 and C5 centrifugal neurons, which have dendrites at several levels in the inner and outer medulla and provide terminal-like swellings at the medulla’s distal surface and within the lamina, where they are presynaptic (Datum et al., 1986). These neurons are known to be γ-aminobutyric acid (GABA)-ergic (Datum et al., 1986; Sinakevitch et al., 2000), and the C2 neuron is known to be selective for motion orientation, but not direction (Douglass and Strausfeld, 1995).

Figure 9A illustrates a novel type of neuron, the morphology of which suggests that it is a feedback neuron from the lobula to the medulla. Its axon is one of several that make up a discrete tract that can be traced to the anterior inner edge of the medulla from the proximal surface of the lobula (Fig. 9D,E), where it provides a dense feltwork of processes through almost all its levels, except for the T5 layer (Fig. 9A). This dendritic organization is readily distinguished from the otherwise columnar appearance of this neuropil (Fig. 9A). The cell bodies of these lobula-to-medulla centrifugal neurons lie anterior and medial to the lobula. The axon leaves the lobula, extends for a short distance medially toward its cell body, and then curves through 180° to project laterally into the anterior side of the medulla (Fig. 9E). Bodian preparations reveal that the region of the lobula representing the front one-third of the visual field contains many fine processes that are suggested to belong to between six and eight of these neurons. Significantly, these are not sex-specific neurons; this feltwork of processes has been identified in both sexes, as have its medulla terminals, and the neuron recorded here is from a female fly. The dendritic branches of this filled neuron extend across approximately 20 retinotopic columns and occupy an area that corresponds to a 30–45°-wide frontal-and downward-looking field of view.

The terminal of this cell in the medulla consists of an extended field of beaded processes, similar to others that invade the same level but that originate from regions of the lateral protocerebrum (Fig. 10A,B). Interestingly, feedback to the inner medulla is also provided by regular...
arrays of retinotopic processes that extend across the second optic chiasma from the lobula plate (Fig. 10C). These centrifugal tangential endings suggest that the inner medulla may be the location at which the activity of a number of retinotopic inputs to the lobula, including Tm, T2, and Y19 cells discussed above, is modulated by reafferent signals.

Responses of the lobula-medulla centrifugal neuron to motion and flicker stimuli are illustrated in Figure 11. The background activity of this neuron was variable, characterized by vigorous spiking and occasional, brief (~1 second) periods when spiking was almost completely inhibited. The spiking was also accompanied by quasiperiodic hyperpolarizations in the baseline membrane voltage. These hyperpolarizations occurred approximately every 12–13 seconds and may have been associated with respiratory or other muscular activity. The responses to edge motion, sinusoidal grating motion, and flicker are described in the order in which they occurred during the experiment.

Motion of single edges across the stimulus screen (Fig. 11A–D) was accompanied by dramatic increases in spiking activity, except during the aforementioned periods when background spiking was being inhibited by mechanisms unrelated to the stimulus. The responses to edge motion occurred whether the edge was brighter or darker than the background intensity of the stimulus monitor, and showed no selectivity for the direction of edge motion. The clearest responses occurred at specific times as each edge proceeded across the stimulus monitor, indicating a receptive field that was positioned frontally and ventrally to the equator, near the bottom edge of the stimulus display. This edge-sensitive region corresponds to the retinotopic region occupied by the cell’s arborizations in the lobula and medulla.

Despite this neuron’s clear responsiveness to edge motion, it showed no convincing evidence of sensitivity to grating motion. Grating motion stimuli were presented at eight different directions and with speeds varied sinusoidally between 0°/second and 200°/second (data not shown). Because the neuron continued its background spiking activity during this time, and subsequently responded to flicker (below), the absence of responses is not attributable to deterioration of the preparation.

Both On and Off phases of wide-field flicker resulted in brief inhibition of the background spiking activity (Fig. 11E,F). No difference was discernible between the responses to light On or Off except that, possibly, the aver-
Fig. 8. Intracellularly filled type 19 Y cell and its responses to flicker and motion. A: Confocal reconstruction from serial sections, showing this neuron's cell body behind the lobula plate (LoP) and arborizations in the inner medulla; in deep, vertically sensitive layers of the lobula plate; and at two of the deepest levels of the lobula (Lo). Dashed lines in the medulla indicate the serpentine layer separating the inner medulla (i Me) and outer medulla (o Me). B–D: Magnified views of A. E: Transient On-hyperpolarizations and Off-depolarizations during wide-field flicker. F: Expanded view from E. G: Spike-like depolarizations in response to grating motion at eight directions. H: Polar plot of integrated responses to grating motion, obtained as in Figure 6. Arrow shows the mean vector (mean angle = 72°), calculated by summing the individual response vectors. Scale bar in A = 20 μm. Scale bar in D = 10 μm (also applies to B and C).
Figure 9
age duration of Off inhibition was slightly greater. Nonetheless, during fast flicker at rates of up to 100 Hz, some spiking persisted. Given the inhibitory effects of light On and Off, the continued spiking suggests that this cell is relatively insensitive to the faster flicker rates.

The edge-motion stimuli also contained a flicker component. Before and after presentation of each edge motion, the screen intensity returned to a standard background intensity that was intermediate between the intensities of the bright and dark edges (Fig. 11A–D, lower traces). At times when the background spike rate was sufficiently high, these flicker-like increases and decreases in overall screen brightness often resulted in momentary inhibition of spiking (Fig. 11B–D, asterisks), consistent with the responses to pure flicker.

**DISCUSSION**

**Cell identities and possible behavioral roles**

This account describes the morphology and physiological responses of retinotopically organized visual interneurons that provide afferent inputs to, or feedback from, the inner lobula. The results include recordings from three previously unknown cell types and provide, with the possible exception of T2, the first descriptions of physiological response properties of these cells. Although intracellular staining of T2-like neurons has been reported on two previous occasions from *Sarcophaga*, both involved costaining of inner medulla amacrine cells in such a way that the identity of the recorded neuron(s) was uncertain (Gilbert et al., 1991). Moreover, in both of those intracellular recordings, the responses to motion differed substantially from the orientation-selective, spike-like depolarizations observed in the present work. In one case (Gilbert et al., 1991; their Fig. 13), responses to grating motion were dominated by sustained rather than transient depolarizations; in the other (Gilbert et al., 1991; their Fig. 21), only small changes from the background voltage fluctuations were observed during motion. Neither recording showed any evidence for orientation or direction selectivity. It thus appears likely that the earlier recordings reflected the activity of amacines rather than T2 cells.

Because all of the small-field neurons in the present study were recorded from male flies and have frontal receptive fields that lie either within or near the male-specific acute zone (Gilbert and Strausfeld, 1991; Strausfeld, 1991), it is possible that aspects of their functional or anatomical properties are sex specific and tailored in some way to serve the visually guided chasing behavior of male flies (Collett and Land, 1978). At present, however, male-specific neurons are known only from the anterior part of the lobula, whereas accounts of small-field retinotopic neurons to the lobula describe these as originating at any retinotopic column across the medulla. Alternative, non-sex-specific roles for neurons with frontal receptive fields might include those that mediate object avoidance or landing responses (Braitenberg and Taddei Ferretti, 1966; Wagner, 1982), both of which can make use of information about the directions or orientations of edge motions. Landing, for example, requires a behavioral decision to override motion-dependent signals that would normally promote stabilized forward flight or initiate a collision-avoidance response. The medulla-lobula neurons, which Bodian-stained preparations suggest are present in both sexes, are particularly interesting in this regard, in that their dendritic fields in the lobula, with feedback connections to the inner medulla, provide them with the potential to modify all frontal inputs to optomotor and/or collision detection circuitry. Whether this modification involves inhibition directly is not known. However, there are no GABAergic correlates of lobula-to-medulla feedback neurons, although there are many GABAergic profiles within the inner medulla (Sinkewitch et al., 2000). If the feedback neurons do inhibit medulla efferents, then this is likely to occur via intervening GABAergic amacines.

**Are functional generalizations possible based on morphology?**

The recordings from transmedullary cells (Figs. 2, 3) show little difference between their responses to flicker and their responses to motion, and no changes appear to be correlated with motion direction or orientation. These results are consistent with a handful of previous recordings from Tm cells, the axons of which project through the T5 layer of the lobula to terminate in deeper layers. In some instances, responses to flicker and motion stimuli may be distinct (Gilbert et al., 1991), but, with the exception of Tm1 cells, which terminate exclusively within the T5 layer of the lobula and exhibit a subtype of directional selectivity (Douglass and Strausfeld, 1995), Tm recordings to date have demonstrated sensitivity to motion but without selectivity for either motion direction or orientation (Gilbert et al., 1991; Douglass and Strausfeld, 1998). Thus, it is tempting to suggest that small-field Tm cells as a group might have little to do with conveying information about motion and perhaps are more concerned with processing other aspects of visual inputs.

The available physiological data, from those retinotopic Y cells which originate from cell bodies that lie beneath the inner medulla, suggest a second possible generalization about anatomically related neurons. Only five distinctive morphological types have been identified for this class of neuron, and intracellular responses have been obtained from three of the five. These are a Y-cell with a moderately wide dendritic field, termed CY2 (Douglass and Strausfeld, 1996), the Y18 cell (Douglass and Strausfeld, 1998), and the Y19 cell described here. In all three cases (although not in an apparent Y18 variant reported by Gilbert et al., 1991), this class of Y cell shows strong selectivity for the direction of grating motion. All other Y cells are known collectively as *TmY cells*, because they arise from cell bodies that lie beneath the inner medulla.
Fig. 10. Tangential and centrifugal organization in the inner medulla. 
A: Many types of beaded endings terminating in the inner medulla (large brackets in A–C) omit the T4 layer (small bracket) or send only occasional processes down among T4 dendrites (inset). 
B: Other types of endings send beaded processes through all levels of the inner medulla, and the trajectories of these processes reflect the T4 layer (small bracket). The distance of this inner region from the lamina afferents is evidenced by the L1 and L2 monopolar cells in B supplying the outer medulla. 
C: Isomorphically arranged axons extend from the lobula plate (LoP), here shown with cobalt-silver-stained horizontal cell dendrites. These axons (two are indicated with arrows) provide varicose branches specifically within the T4 dendritic layer (small bracket). Scale bars in B = 25 μm for A–C; bar in inset = 5 μm.
bodies above the outer surface of the medulla. Recordings to date from such TmY cells (Gilbert et al., 1991; Douglass and Strausfeld, 1998; and unpublished observations) have provided no evidence for direction or orientation selectivity.

The data from Tm cells and TmY cells seem to suggest that developmentally and/or morphologically related neurons might have similar functional properties. Certainly this is true in some instances, as in the case of optic-flow-sensitive HS and VS tangential cells in the lobula plate, but such generalizations may be unreliable. Although morphologically similar neurons may share a common evolutionary and functional ancestry, their present-day functional roles easily could have diverged. In conclusion, additional recordings from Y cells, TmY cells, and Tm cells are required to determine which members of these groups are functionally related. Developmental studies of clonal lineages (Lee and Luo, 2001) would be a useful corollary to such studies.

Morphological bases for functional properties

In any spatially mapped neuropil, individual neuronal morphologies and the synaptic relationships among them offer numerous possibilities for shaping spatial properties within integrative circuits that subextend geometrically precise arrangements of sensory inputs. Nevertheless, it is surprisingly difficult to demonstrate specific morphological correlates of functional properties. In 1962, Hubel and Wiesel proposed two simple yet compelling models to explain how selectivity for bar orientation could arise in the “simple” and “complex” orientation-selective neurons in the visual cortex of cats. In both models (Hubel and Wiesel, 1962), the spatial distribution of inputs from the lateral geniculate nucleus (LGN) is spatially similar to the stimulus. Although physiological evidence now confirms and amplifies these basic ideas with regard to the LGN inputs (Reid and Alonso, 1995; Alonso et al., 2001), and general patterns of intracortical connections have been linked with orientation preferences (Gilbert and Wiesel, 1989; Malach et al., 1993; Sincich and Blasdel, 2001), there is still no direct morphological observation that distinguishes the orientation of orientation-sensitive cells. In another intensively studied system, years of careful scrutiny have yet to reveal any morphological correlate to the preferred directional sensitivities of rabbit retinal ganglion cells (Amthor et al., 1989; Oyster et al., 1993; Yang and Masland, 1994; He and Masland, 1998).

Are the morphological bases for functional properties invariably cryptic? The optic lobes of flies suggest otherwise. Historically, the orientation of dendrites has been
taken to suggest orientation and directional preferences, as in the case of HS and VS neurons (Pierantoni, 1976), which later were shown to have, generally, horizontal- and vertical-motion sensitivity (Hausen, 1976; Hengstenberg, 1982). In calliphorid flies, the axon collaterals of L4 monopolar cells projecting from the lamina to the medulla are arranged in an asymmetric, rectilinear pattern that has been proposed as a possible substrate for elementary motion detection or lateral inhibition along three directions across the retinotopic mosaic (Braitenberg and Hauser-Hohlschuh, 1972). The dendritic trees of T5 bushy T cells also show characteristic asymmetries (Strausfeld and Lee, 1991; Buschbeck and Strausfeld, 1996), suggesting a possible relationship to mechanisms that give rise to directional sensitivity in T5 neurons (Douglass and Strausfeld, 1995). Perhaps the most spectacular example of morphological asymmetry in the optic lobes occurs in the type 17 Y cell (Strausfeld, 1976), with its long, unilateral branches in the medulla, lobula, and lobula plate that all “point” in the same retinotopic direction (Douglass and Strausfeld, 1998). In none of these examples, however, has a direct link been demonstrated between the morphology and the functional properties of single cells.

The close correspondence between the preferred orientation of the dendrites of T2 within the outer medulla and the preferred motion orientation is the first suggestion that this cell's physiological activity may be directly related to its dendritic morphology. T2 presumably, based on the dimensions of its dendritic branches, receives inputs not only from its parent retinotopic column but also from adjacent columns that lie along T2's preferred dendritic axis of orientation (Fig. 12A). The basic principle of mapping edge-sensitive activity from a retinotopically defined axis onto a single neuron, is the same as that originally proposed by Hubel and Wiesel (1962) to explain orientation-sensitive properties of the simple cells in feline visual cortex. One point of difference is that Hubel and Wiesel envisioned presynaptic cells with lateral branches converging onto the orientation-selective neuron, whereas T2 morphology suggests an arrangement in which the lateral branches belong to the postsynaptic cell. A more significant difference involves the relationship between the preferred morphological axis and the physiological preferred orientation. The Hubel and Wiesel model (see Fig. 19 in Hubel and Wiesel, 1962) postulates that simple cells prefer edges that are parallel (with motion perpendicular) to a row of retinotopic inputs. In contrast, the present data from T2 show a preference for edges that are perpendicular (with motion parallel) to the preferred dendritic axis. These observations suggest that distinct types of mechanisms can generate orthogonal preferred orientations based on the same underlying spatial bias in morphology.

Figure 12 illustrates a possible mechanism for the type of orientation selectivity that we have observed in T2. Excitatory inputs arising from L1 monopolar cells and reaching T2 from three adjacent retinotopic columns would be modulated by a graded field of inhibitory inputs that is stronger at one end of T2's elongated dendritic field and weaker at the other end (Fig. 12B,C). With this arrangement, the relative timing of excitatory and inhibitory inputs can vary in such a way that only edge motion along T2's preferred orientation results in threshold crossings (Fig. 12D). A possible source of the required inhibitory inputs for T2 is suggested by systems of centrifugal cells within each column, which GABA immunocytochemistry suggests to be inhibitory (Sinakevitch, unpublished data from *Phaenicia*).

**Insights into motion-processing circuitry**

The results of this study provide several new insights into the functional organization of motion-processing cir-
circuitry in dipterous insects, especially with regard to sources of motion-specific information in the lobula, motion-specific feedback from the lobula and lobula plate to the inner medulla, and a possible hierarchical organization of motion-detecting circuitry (Higgins et al., 2001). The presence of numerous spiny processes in the arborizations of Y19 at the level of the medulla and lobula plate (Fig. 8) suggests that these branches are mainly receptive, postsynaptic sites, whereas the varicose branches in the deep lobula are consistent with presynaptic sites. In combination with the physiological responses to motion, these observations lead us to propose that Y19 acquires its direction-selective activity from the lobula plate and/or inner medulla and conveys direction-selective activity to two of the three additional direction-sensitive Y neurons discussed above, we can now state with some certainty that the lobula is well supplied, and at several depths, with direction-selective motion information that originates in the lobula plate or inner medulla. Meanwhile, the responses of T2 clearly demonstrate that orientation-selective information is supplied to the lobula directly from the medulla.

Together these observations support several basic conclusions about the nature of dipterous motion-processing pathways. First, direction-selective (Gilbert and Strausfeld, 1991) or, in dragonflies (O’Carroll, 1993), orientation-selective responses of lobula neurons need not arise from hypothetical motion-detecting circuits that are intrinsic to this neuropil. Instead, these properties of motion selectivity can be accounted for by known inputs from the medulla and lobula plate. Second, the list of parallel small-field motion-selective pathways that originate in the medulla, heretofore covering direction-selective (Tm1–T5; Douglass and Strausfeld, 1995) and non-direction-selective (iTm–T4; Douglass and Strausfeld, 1996) pathways to the lobula plate, now includes an orientation-selective pathway to the lobula. The presence of several distinct motion-selective pathways at this level is consistent with a proposal (Higgins et al., 2001) that all three channels are supplied divergently by a single, more peripheral assemblage of elementary motion-detection circuits.

A further indication that there is a peripheral divergence of motion pathways is suggested by the organization of the outer dendrites of T2 cells. These are disposed above the L2 monopolar cell endings (Fig. 4E) but could receive direct inputs from the outer swelling of the L1 monopolar cell terminal. This arrangement might suggest a fundamental point of divergence in each retinotopic pathway whereby L2 neurons, along with T1 afferents ending at the same level (Fig. 4J,K), are presynaptic to the intrinsic Tm cell iTm (see Douglass and Strausfeld, 1995) and to the type 1 Tm (Tm1, iTm ends in the inner medulla at the level of T4 neurons, which extend as quartets from each column into levels of the lobula plate. Tm1 neurons end in the T5 layer of the lobula, which provides quartets of directionally selective, motion-sensitive neurons to the same levels of the lobula plate.

Thus, L2 is associated with neurons that supply the lobula plate, whereas L1, assuming that it makes functional connections with T2 neurons, supplies channels to the lobula that encode motion orientation but not direction. The occurrence of pairs of T2 neurons, in which only one of the pair has dendrite-like processes in the inner medulla, suggests that, in each T2 pair, one member only might be modulated by afferents supplying the inner medulla, such as the lobula-to-medulla centrifugal cell. Such an arrangement would be reminiscent of the L2-to-lobula plate pathway via T4 and T5 cells (Strausfeld and Lee, 1991; Bausenwein and Fischbach, 1992). The dendrites of T4 quartets in the inner medulla are disposed at levels invaded by afferents as well as centrifugal processes from deeper regions. In contrast, quartets of T5 cell dendrites are not similarly invested by centrifugal processes, suggesting that feedback modulation affects only one of these parallel channels, namely, that composed of T4 cells.

Behavioral tests have shown that dipterous insects (Campbell and Strausfeld, 2001), such as Hymenoptera (Srinivasan, 1994), can visually discriminate patterns based on the orientations of contrasting edges. Campbell and Strausfeld (2001) considered several alternative mechanisms that might explain this capability in flies and concluded that the most likely, as was originally proposed with reference to bees (Srinivasan, 1994; Chandra et al., 1998), would involve arrays of small-field, orientation-selective neurons, each broadly tuned to one of three preferred axes that differ by 120°. Recordings from unidentified orientation-selective neurons with wide receptive fields in the bee optic lobe led Yang and Maddess (1997) to postulate the presence of similar small-field arrays supplying these wide-field elements. In fact, McCann and Dill (1969) had already proposed small-field arrays that would supply orientation-selective units which they discovered in extracellular recordings from the fly optic lobe, and which they attributed to the medulla based on careful positioning of the electrodes. The recordings from T2 now identify an anatomical substrate for these arrays, and implicate T2 as an essential element of orientation-detecting circuitry in flies. Moreover, the correlation between physiological and morphological preferred axes of orientation in T2 now makes it possible to test the hypothesis that the preferred orientations of individual small-field units are clustered around a minimum of three axes.

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LITERATURE CITED


