Structure of the Vertical and Horizontal System Neurons of the Lobula Plate in *Drosophila*

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ABSTRACT

The lobula plate in the optic lobe of the fly brain is a high-order processing center for visual information. Within the lobula plate lie a small number of giant neurons that are responsible for the detection of wide field visual motion. Although the structure and motion sensitivity of these cells have been extensively described in large flies, the system has not been described systematically in *Drosophila*. Here, we use the mosaic analysis with a repressible cell marker (MARCM) system to analyze a subset of these cells, the horizontal and vertical systems. Our results suggest that the *Drosophila* horizontal system is similar to those described in larger flies, with three neurons fanning their dendrites over the lobula plate. We found that there are six neurons in the *Drosophila* vertical system, a figure that compares with 9–11 neurons in large flies. Even so, the *Drosophila* vertical system closely resembles the systems of larger flies, with each neuron in *Drosophila* having an approximate counterpart in large flies. This anatomical similarity implies that the inputs to the vertical system are similarly organized in these various fly species, and that it is likely that the *Drosophila* neurons respond to motions similar to those sensed by their specific structural counterparts in large flies. Additionally, the similar appearance of vertical system cells in multiple cell clones demonstrates that they share a common developmental lineage. Access to these cells in *Drosophila* should allow for the use of genetic tools in future studies of horizontal and vertical system function. J. Comp. Neurol. 454:470–481, 2002. © 2002 Wiley-Liss, Inc.

Indexing terms: dendrites; optic flow; optic lobe

In order to navigate through their complex natural habitat, many flying insects rely heavily on vision. Particularly important for flight control is information on the movement of the fly's body itself. When a fly changes orientation or moves in some direction, its entire visual field shifts in a manner specific to the maneuver being performed (Koenderink and van Doorn, 1987). For example, as an insect engages in straight level flight, objects in its surroundings flow from the front to the back of its visual field. In dipteran insects, the integration of such inputs is carried out largely by giant neurons of the lobula plate in the lobula complex (Dvorak et al., 1975).

These neurons have been characterized in great anatomical and physiological detail in various large flies. Initial anatomical analyses of the lobula plate in the housefly (*Musca domestica*) revealed two systems of giant neurons, which were termed the horizontal system (HS) and vertical system (VS) (Pierantoni, 1976; Strausfeld, 1976a). The horizontal system comprises three giant neurons, HS north (HSN), HS equatorial (HSE), and HS south (HSS), whose overlapping dendrites fan out over the dorsal, middle, and ventral parts of the lobula plate, respectively. The

vertical system in the housefly was shown to have nine cells, each of which has a dendritic arborization covering a band stretching out along the dorsal-ventral axis of the lobula plate. The axons of these cells extend to the posterior slope in the periesophageal region, where they synapse onto the ocellar fibers and a branch of a huge fiber of the cervical connective (Strausfeld, 1976a; Heisenberg et al., 1978). Detailed descriptions in the blowflies *Calliphora erythrocephala* (Hausen et al., 1980; Hengstenberg et al., 1982) and *Phaenicia sericata* (Eckart and Bishop, 1978) indicate that the vertical systems in these species have 9–11 cells with structures similar to their counterparts in *Musca*.

The fly visual system is organized as a retinotopic map, whereby the visual field is represented spatially as input into a given layer of the optic lobe (Strausfeld, 1976b). This map persists through the first and second optic neuropils, the lamina and medulla, and through the input to the lobula complex, comprising the lobula and the lobula plate. However, output of the vertical system and horizontal system from this neuropil is not organized into a retinotopic map (Strausfeld, 1976b). These facts imply that the conversion from the input of spatially organized visual information to output relating to specific wide-field stimuli takes place in part in these cells. In support of this idea, studies have shown that activity in discrete anterior-posterior layers of the lobula plate correspond not to regions of the visual field, but to directions of optic flow in the visual field as a whole. Specifically, four layers are active in response to front to back (most anterior layer), back to front (middle anterior), upward (middle posterior), and downward (most posterior) motions (Buchner and Buchner, 1984; Buchner et al., 1984).

This schematic organization of the input to the lobula plate agrees well with experiments that have characterized the function of the vertical system in vivo. In numerous experiments on blowflies, VS neurons have been shown to depolarize in response to wide-field motion in their preferred direction. In its simplest form, this stimulus can be a downward moving pattern of horizontal stripes (Hengstenberg, 1977; Soohoo and Bishop, 1980; Hengstenberg, 1982; Single and Borst, 1998). Certain VS neurons have also shown responses to horizontal shifts in the visual field (Eckert and Bishop, 1978; Eckert, 1982). These observations and others led to models in which certain neurons in the lobula and lobula plate act as detectors of wide-field motions in the visual field (Egelhaaf et al., 1988; Borst and Egelhaaf, 1989; Egelhaaf and Borst, 1993). In accordance with these models, a more extensive analysis has indicated that VS neurons respond most robustly to wide-field shifts that would accompany flight motions such as changes in pitch or roll (Krapp et al., 1998). It is therefore postulated that the vertical system functions in sensing self-motion during flight.

Although the horizontal and vertical systems have been characterized extensively in the housefly and blowfly, structural information on these cells in *Drosophila* is somewhat sparse. In terms of cellular composition, the *Drosophila* horizontal system appears to be similar to those in large flies, with three cells extending their fan-shaped dendrites through the lobula plate (Heisenberg et al., 1978; Fischbach and Dittrich, 1989). The vertical system is simpler than those of the housefly and blowfly, with only five to seven cells (Heisenberg et al., 1978). Golgi staining in *Drosophila* has shown the VS neurons to be

generally similar to those described in larger flies (Fischbach and Dittrich, 1989; Buschbeck and Strausfeld, 1997), but the system as a whole has yet to be described in detail. In the current study, we report the general structures of the HS and VS neurons in the lobula plate in *Drosophila* and provide a detailed analysis of the dendritic organization of individual VS neurons.

MATERIALS AND METHODS
Fly culture, histology, and microscopy

Drosophila melanogaster were grown on standard media at 25°C. During clonal analysis, larvae hatched over a 2-hour interval were moved to plastic vials containing approximately 10 ml of food. Larvae were kept at a concentration of 80 per vial. Mitotic recombination was induced via heat shock (40 minutes in a 37°C water bath, 30 minutes at room temperature, 40 minutes in 37°C water bath) at 2 and 3 days after hatching. Adult female flies between 2 and 5 days after eclosure were dissected, fixed, and stained as described (Lee and Luo, 1999). Briefly, dissected brains were fixed in 4% paraformaldehyde in 100 mM phosphate buffer (pH 7.2) for 30 minutes at room temperature and then washed 6 × 10 minutes in phosphate buffer (pH 7.2) with 0.1% Triton X-100 (PBT). They were then incubated in PBT with 5% normal goat serum for 30 minutes and stained with 100× diluted rat anti-mCD8-αchain (Caltag Laboratories. Burlingame, CA) overnight at 4°C. They were washed 6 × 10 minutes in PBT and then incubated for 2 hours with 500× diluted goat anti-rat CY3-conjugated antibody (Jackson ImmunoResearch, West Grove, PA) in PBT. A final 6 × 10-minute washes in PBT preceded mounting using Slow-Fade (Molecular Probes, Eugene, OR).

A Bio-Rad (Hercules, CA) MRC 1024 laser scanning confocal microscope and the Laser Sharp image collection program were used. Images were processed using Adobe Photoshop. Three-dimensional traces of the dendrites were produced from confocal stacks using MicroBright-Field Neurolucida software. Briefly, dendritic branches were traced such that turning points, branch points, and endpoints were specified in X, Y, and Z positions within the confocal stack. These points were then combined to provide the overall shape of the dendritic arborizations. Traces were then rotated using Rotator 3.5 software.

Fly strains and the MARCM system

The Gal4-3A line was isolated in an enhancer-trap screen for lines that show specific expression patterns in the adult brain (T.R., unpublished data). P[Gal4] lines were generated as described (Brand and Perrimon, 1993) and crossed to the UAS (Upstream Activating Sequence) -tau reporter (Ito et al., 1997b). The progeny were analyzed by mass histology (Heisenberg and Bohl, 1979) and immunohistochemistry with an anti-Tau antibody. Once the Gal4-3A driver was isolated, its nonclonal expression pattern was characterized in a *UAS-mCD8-GFP / + ; GAL4-3A / +* female.

The MARCM system requires that the GAL80 gene, a repressor of GAL4-mediated expression from a UAS, be eliminated from a cell or clone of cells, thus allowing for the expression of a UAS-driven marker gene (Lee and Luo, 1999). Elimination of GAL80 is allowed by the inclusion of Flp recombinase target (FRT) proximal to GAL80 on the
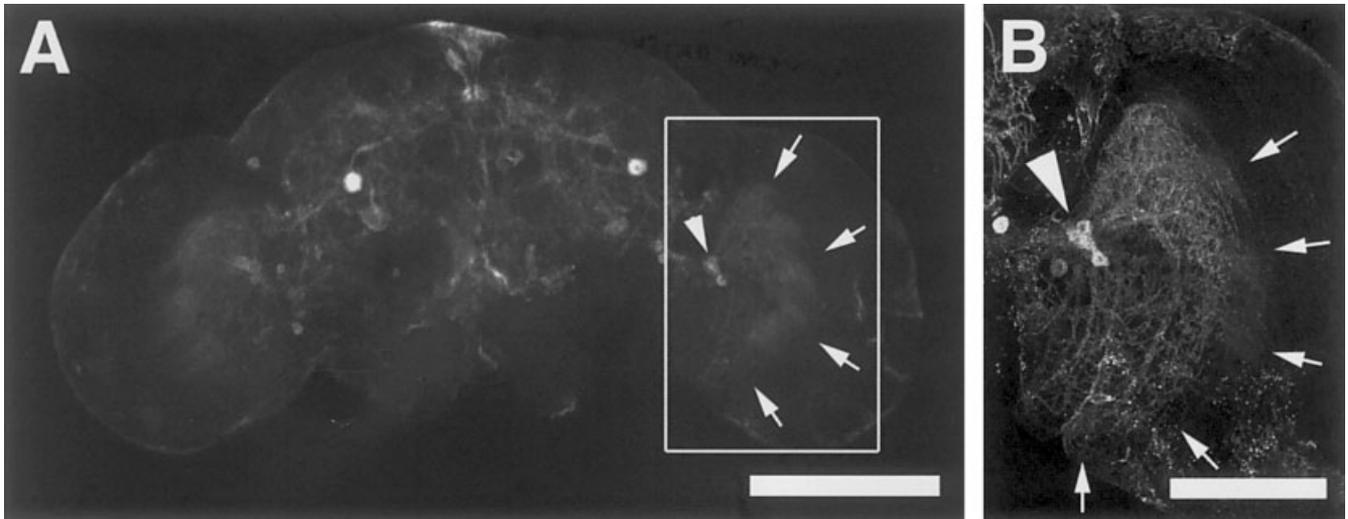
Fig. 1. Expression pattern of Gal4-3A. **A:** Posterior view of the entire brain. The cell bodies (arrowhead) and dendrites (arrows) of the VS neurons can be seen. Other cells and their neurites, which have not been characterized, are visible, particularly in the protocerebrum. The neurons of the horizontal system are too faint to be seen in a nonclonal analysis. They are only visualized when MARCM is performed. The box shows the area magnified in B. **B:** Close-up of the optic lobe showing the position of the vertical system dendrites (arrows) and cell bodies (arrowhead). Scale bar = 100 μm in A; 50 μm in B.

chromosome being used for recombination and on the homologous chromosome. The *flp* gene is included and driven by a heat shock promoter, thus allowing for heat shock-induced mitotic recombination at the FRT sites. The resulting elimination of GAL80 in this cell or clone of cells allows the driver (Gal4-3A), to cause expression of the membrane localized marker, *mCD8-GFP* (Lee and Luo, 1999), which is later used to visualize the cell or cells. The limited expression pattern of Gal4-3A prevents the marking of a large number of background clones. This results in a system that allows for the positive marking of a cell or clone of cells in a dark background. Such positive staining is critical for the analysis of fine cellular structures such as dendrites.

Inverse PCR showed the Gal4-3A driver to be located at 66A on 3L. Accordingly, the following female genotypes were created for the analysis using MARCM: *tubP-GAL80, hs-flp, FRT^{19A} / yw, FRT^{19A} ; UAS-mCD8-GFP / + ; GAL4-3A / +* for recombination on X, *hs-flp, UAS-mCD8-GFP / + ; tubP-GAL80, FRT^{40A} / FRT^{40A} ; GAL4-3A / +* for recombination on 2L, *hs-flp / + ; FRT^{G13}, tubP-GAL80 / FRT^{G13}, UAS-mCD8-GFP ; GAL4-3A / +* for recombination on 2R, and *hs-flp, UAS-mCD8-GFP / + ; tubP-GAL80, FRT^{2A} / GAL4-3A, FRT^{2A}* for recombination on 3L.

RESULTS
The Gal4-3A driver

The lobula complex is in the most medial portion of the optic lobe and comprises the anterior lobula and the posterior lobula plate. A previous analysis of the *Drosophila* lobula plate has revealed three systems of giant neurons: the vertical system with five to seven cells, the horizontal system with three cells, and the two M-cells (Heisenberg et al., 1978). To describe the fine structural details of these cells, we were interested in visualizing them using the MARCM system, which depends on a tissue-specific Gal4 driver (Lee and Luo, 1999). The Gal4-3A driver shows expression in a limited number of cell types throughout the brain (Fig. 1A). Within the optic lobe, the cells of the vertical system are notable among those expressing Gal4-3A, but a generally high background of marked cells indicates that Gal4-3A is expressed in cells other than the VS neurons (Fig. 1).

Giant neurons of the *Drosophila* lobula plate

The MARCM system in *Drosophila* allows for the marking of individual cells or groups of clonally related cells in an otherwise unlabeled background (Lee and Luo, 1999). We have used this system in conjunction the Gal4-3A driver to label the HS and VS neurons of the lobula plate. Using MARCM, we can label the cells of each of these systems in great structural detail. Although the HS and VS neurons are the most prominent among the clones that we create using Gal4-3A, a few other cell types appear in the optic lobes and elsewhere in the brain (data not shown).

Because mitotic recombination can lead to Gal4-UAS activity either in a dividing neuroblast or in a ganglion mother cell, clones with different numbers of cells form (Fig. 2A). When a neuroblast loses the Gal80 repressor as a result of recombination early in development, all the progeny of this cell have the potential to be marked (Lee and Luo, 1999). This can lead to a neuroblast clone in which the rest of the of this lineage is labeled. Interestingly, we find that the cells of the vertical system frequently appear in the same neuroblast clone, indicating that they share a common lineage. Such neuroblast clones in the vertical system allow for a characterization of the system as a whole (Fig. 2B).

As in larger flies, the dendrites of the vertical system cover the lobula plate broadly in the dorsal-ventral and
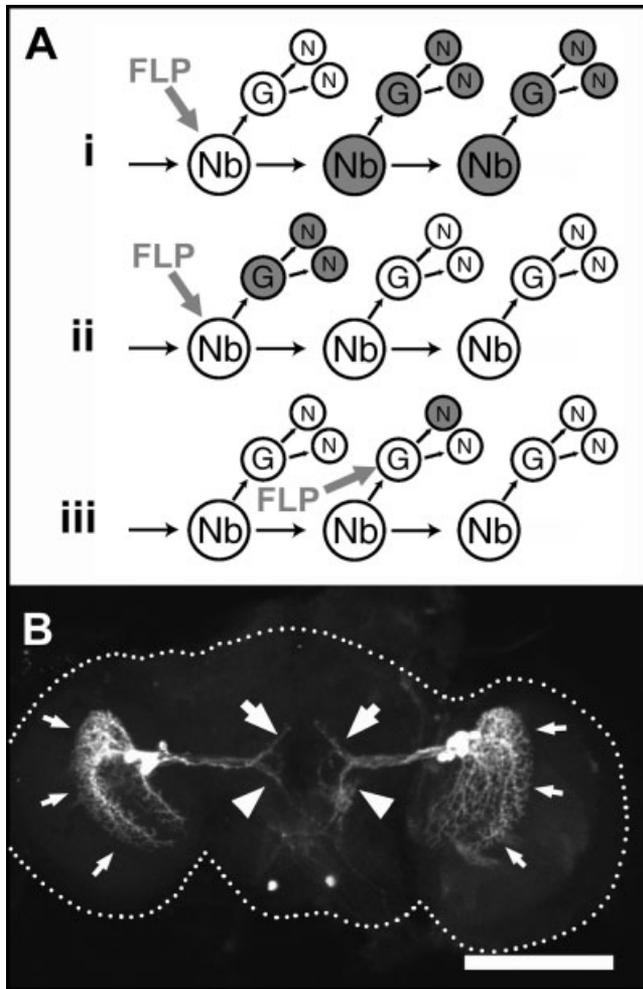
Fig. 2. Clone formation using MARCM. **A:** Schematic diagram showing a typical CNS neuroblast division pattern in *Drosophila*. NB, neuroblast; G, ganglion mother cell; N, neuron. Shaded circles represent those that will be marked as members of the clone lacking GAL80. Mitotic recombination caused by the expression of Flipase (FLP) in a neuroblast can result in an NB clone (i), or a two-cell clone (ii), depending on whether the daughter gaining Gal4-UAS activity is the neuroblast (i) or the ganglion mother cell (ii). Recombination in a ganglion mother cell results in a single cell clone (iii). (Adapted from Lee et al., 1999). **B:** Posterior view of the entire brain. Neuroblast clones containing most or all of both vertical systems can be seen with their dendrites (small arrows) covering the lobula plates dorsoventrally and mediolaterally. The anterior-posterior axis will be described in later figures. The axons extend to the center of the protocerebrum and divide into dorsal (arrows) and ventral (arrowhead) branches. The dotted line outlines the brain. Scale bar = 100 μm.

medial-lateral axes (Fig. 2B). The anterior-posterior structure of these dendrites will be addressed as we describe these cells individually (see Figs. 4–9). The axons of these cells travel medially and terminate near the esophagus (Fig. 2B). The horizontal system comprises three individually identifiable cells extending their dendrites over the dorsal (HSN, Fig. 3A), central (HSE, Fig. 3B), and ventral (HSS, Fig. 3C) lobula plate. Through extensive bifurcation, the dendrites of each of these cells covers a wide area of the lobula plate, resulting in significant overlap of the

dendritic fields of the HS cells. As judged from confocal images, these dendritic fields are quite flat in the anterior-posterior axis. Their axons extend medially and ventrally within the brain in a manner similar to that described in large flies (Strausfeld, 1976a). In all these respects, the HS neurons appear to be similar to those described in *Drosophila* using Golgi staining (Fischbach and Dittrich, 1989).

In addition to the cells of the horizontal system and vertical system, we occasionally see other giant neurons in the lobula plate (data not shown), possibly representing the previously described M-cells (Heisenberg et al., 1978).

Individual neurons in the vertical system

To characterize the complex and overlapping dendrites of the VS neurons, we have imaged and traced single cell clones and observed their dendritic structures in three dimensions. As in other flies' vertical systems, the most complex cells extend their dendrites into the most lateral area of the lobula plate. The outermost cell, VS1, is characterized by a main dendritic shaft that produces one or a few dorsally projecting branches before sweeping ventrally (Fig. 4A). As the main shaft extends ventrally, it continues to produce smaller branches that combine to form a narrow band covering the most lateral part of the lobula plate. Like its counterparts in larger flies, this cell is relatively flat but extends anteriorly in the dorsal aspect of its dendritic tree (Fig. 4D).

The VS2 cell extends its dendrites into a region of the lobula plate that is medial to, but overlapping that of the VS1 cell (see below). Its overall dendritic structure, with a major dendritic shaft sweeping from dorsal to ventral, is similar to that of VS1, but there are a few recognizable differences (Fig. 5). The VS2 cell is less complex, with fewer dendritic branches than VS1. Additionally, it invariably has a single dorsally projecting dendrite, which often extends away from the main dendritic shaft before branching further (Fig. 5A). By comparison, VS1 has dorsal dendrites that branch almost immediately upon leaving the main dendritic shaft (Fig. 4A). Finally, VS2 is extremely flat in the anterior-posterior axis, with all of its dendritic structures among the posterior dendrites of the vertical system (Fig. 5D; also see below).

The central two cells of the vertical system are similar in structure to one another. Compared with VS1 and VS2, VS3 and VS4 are less defined by a dominant dendritic shaft that sweeps from dorsal to ventral (Figs. 6A, 7A). Although VS3 and VS4 cells have a ventrally sweeping dendrite, they also have major dendrites projecting and branching dorsally. This makes these dendritic trees more nearly symmetrical along their dorsal-ventral axis than the other members of the vertical system. Also unlike most other VS neurons, VS3 and VS4 elaborate their dorsal dendrites at different anterior-posterior levels of the dorsal lobula plate (Figs. 6C,D, 7C,D). There are two criteria that allow VS3 and VS4 to be distinguished. First, the dorsal dendrites of VS3 continue to slant laterally as they extend dorsally (Fig. 6A), whereas these dendrites in VS4 curve so that they extend directly dorsally and even slightly medially (Fig. 7A). Second, the dorsal dendrites of VS3 have a major component that extends anteriorly (Fig. 6D), whereas anterior projections are few and simple in VS4 cells (Fig. 7D). Using these criteria, it is generally possible to distinguish between single VS3 and single VS4
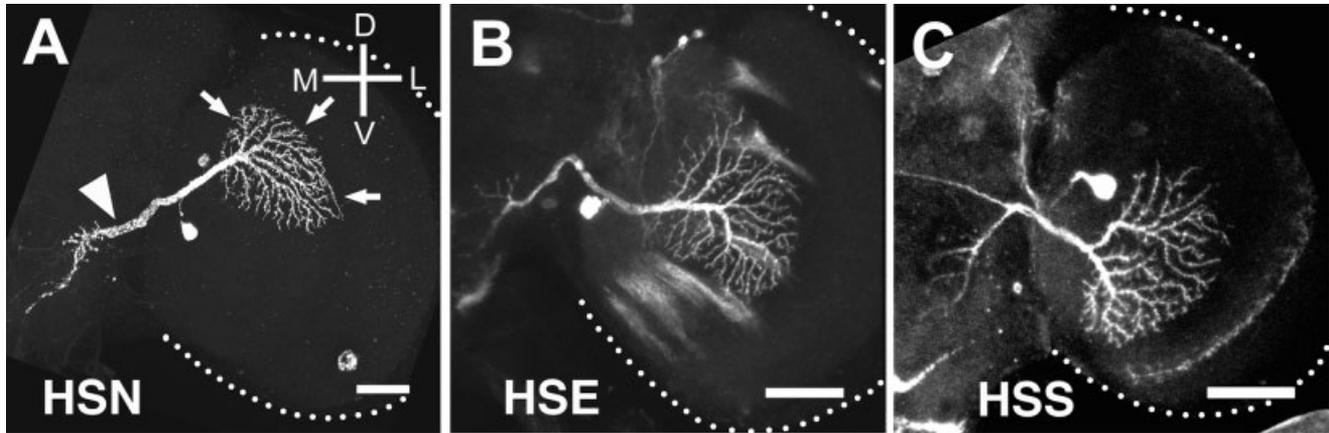
Fig. 3.   The HS neurons. **A:** A single cell clone of an HSN cell. Its dendrites can be seen covering the dorsal aspect of the lobula plate (arrows), and its axon extends medially and ventrally into the proto-cerebrum (arrowhead). **B:** An HSE cell. The dendrites are in the center of the lobula plate dorsoventrally. **C:** An HSS cell. The dendrites are in the ventral part of the lobula plate. Dotted lines show the edges of the optic lobe. D, dorsal; V, ventral; M, medial; L, lateral. Scale bars = 25 μm.
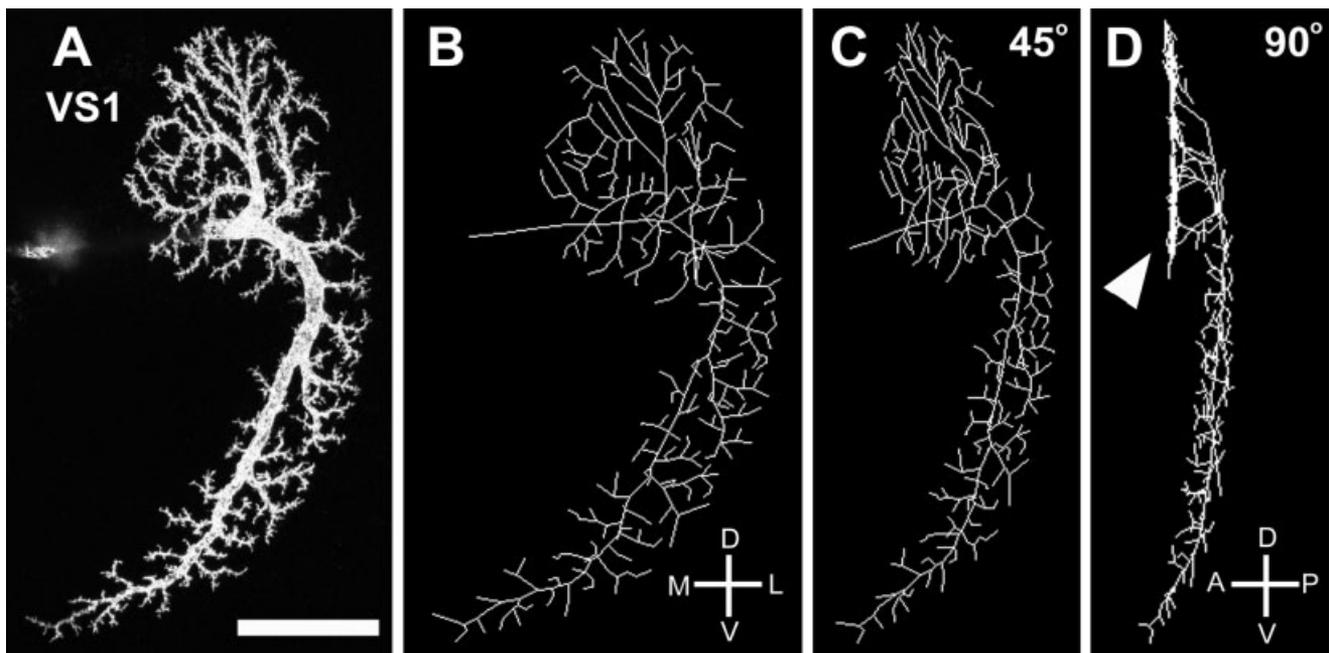

Fig. 4.   The VS1 neuron. **A:** Posterior view of the dendrites of a VS1 neuron. The dendritic tree is dominated by a main dendritic shaft, which sweeps from dorsal to ventral. Branches depart in an approximately radial fashion from this main shaft to cover the characteristic field of VS1. **B:** A three-dimensional tracing of the dendrites from A, viewed from the same perspective. **C:** The same tracing, rotated 45°. **D:** The same tracing, rotated 90° to show anterior extension by the dorsal dendrites (arrowhead). For details on the structure of this cell, see the text. A, anterior; P, posterior. Scale bar = 25 μm and applies to all panels.

cells, but the structures are sufficiently similar that this distinction sometimes cannot be made.

The final two members of the vertical system are notable for branching predominantly in the dorsal lobula plate (Figs. 8, 9). Both VS5 and VS6 have ventral dendrites with relatively little complexity. They can be distinguished based on two characteristics. First, VS5 projects at least two major dendritic branches dorsally, one from the initial dorsal extension, and one from a major branch that initially grows into the central lobula

plate before contributing a dorsally extending branch (Fig. 8A). In contrast, VS6 has a single major dorsal branch that is an extension of the original dorsal arborization (Fig. 9A). Additionally, VS5 sends its dorsal dendrites dramatically anteriorly in the lobula plate, generally 20 μm or more out of the plane formed by the ventral dendrites (as judged from confocal stacks; data not shown). VS6 has anteriorly projecting dorsal dendrites, but they tend to extend approximately 10 μm out of this plane.
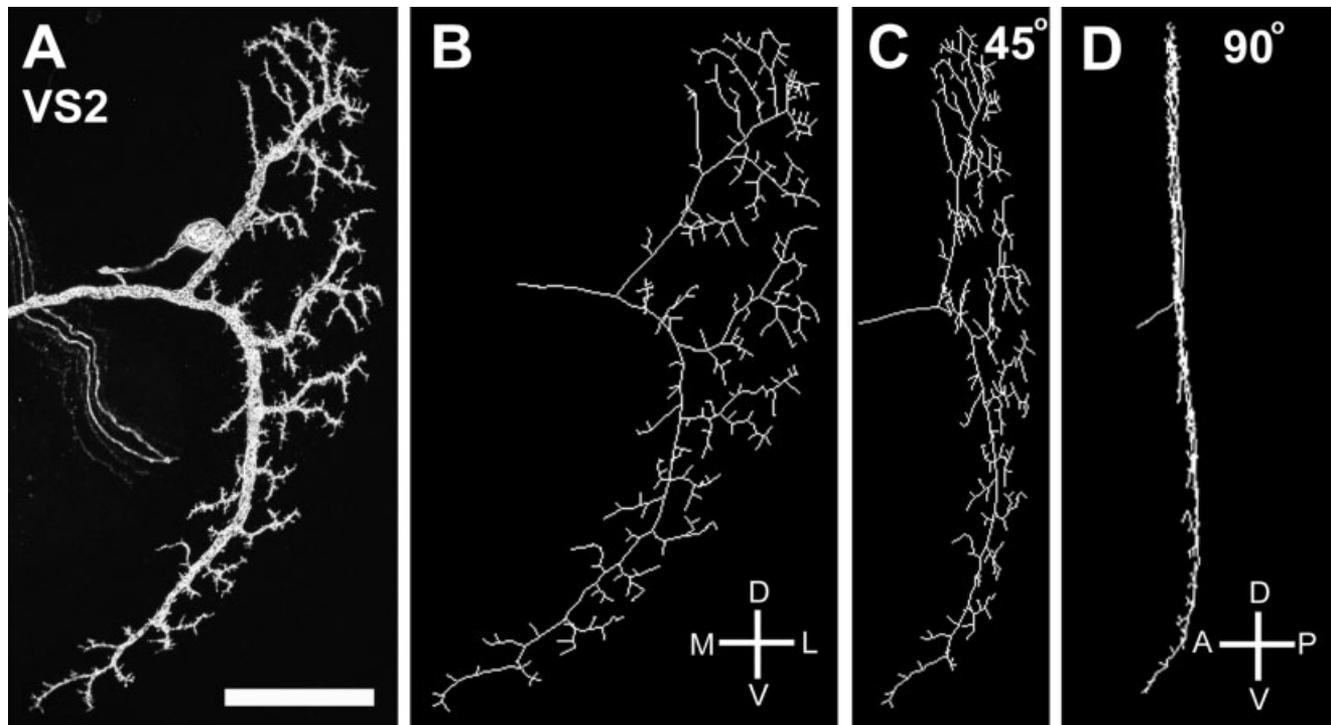
Fig. 5. The VS2 neuron. **A:** Posterior view of the dendrites of a VS2 neuron. The overall shape of the dendritic field is similar to that seen for VS1, but the dendrites are less complex. **B:** A three-dimensional tracing of the dendrites from A, viewed from the same perspective. **C:** The same tracing, rotated 45°. **D:** The same tracing, rotated 90°, shows the dendritic structure to be flat in the anterior-posterior axis. For details on the structure of this cell, see the text. Scale bar = 25 μm and applies to all panels.

Stereotypy in the VS1 neuron

One important property of any cellular system used for the genetic study of dendrites is stereotypy in the dendritic structure from animal to animal. In order to characterize the level of stereotypy among vertical system dendrites, we have analyzed one example, the VS1 neuron, in detail for 12 different animals. Examples can be seen in Figure 10. All VS1 dendrites observed are dominated by a major dendritic shaft that sweeps from the dorsal to the ventral part of the dendritic field. In most cases, this shaft gives rise to smaller branches but is itself unbranched (Fig. 10A–C). Occasionally, there is a bifurcation of this major shaft in the ventral aspect of the field (Fig. 10D).

The other major variability found among VS1 dendrites is the manner in which the dorsal part of the field is innervated. In some cases, a single major branch accounts for all the dorsal dendrites (Fig. 10A,B), whereas other cells have multiple dorsal dendrites (Fig. 10C,D). Despite these two variations in the details of VS1 dendrites, there are important ways in which VS1 dendrites are invariant. The overall three-dimensional dendritic field is almost identical from one animal to another regardless of the specific branching characteristics. This field involves dorsal dendrites that extend anteriorly in the lobula plate and ventral dendrites that remain in the posterior (Fig. 4D; data not shown for dendrites in Fig. 10). Also, the total complexity of the dendritic trees and total length of the dendrites in a given VS1 cell are highly consistent, regardless of the manner in which the dendrite branches (un-

published observations). These observations indicate that, although there are some differences in the structural details of these dendrites from animal to animal, there are important invariant features that should allow members of the vertical system to be used in studies of dendrite development. Our experience with all the members of the vertical system leads us to believe that the level of stereotypy is similar among other cells in the vertical system (data not shown).

Relative position of the vertical system cells

Although looking at these cells individually is necessary for characterizing their structures in detail, we need to consider them as a group in order to characterize the overall dendritic structure of the vertical system. This can be done by analyzing neuroblast clones (Fig. 11). As mentioned previously, the VS neurons as a group send their dendrites over a large medial-lateral and dorsal-ventral field. In the ventral part of the lobula plate, the dendrites of individual cells form distinct but overlapping bands of innervation (Fig. 11A,B). In the dorsal area, the fields of these cells' dendrites overlap more extensively and are difficult to distinguish without information about their anterior-posterior position. Looking at neuroblast clones from various angles reveals that the ventral dendrites of the system as a whole are extremely flat, with little anterior-posterior depth (Fig. 11C). The dorsal dendrites, as we have already shown for individual cells, spread over a much greater anterior-posterior range (Fig. 11C–E). An interesting detail of this factor is that anteriorly extending
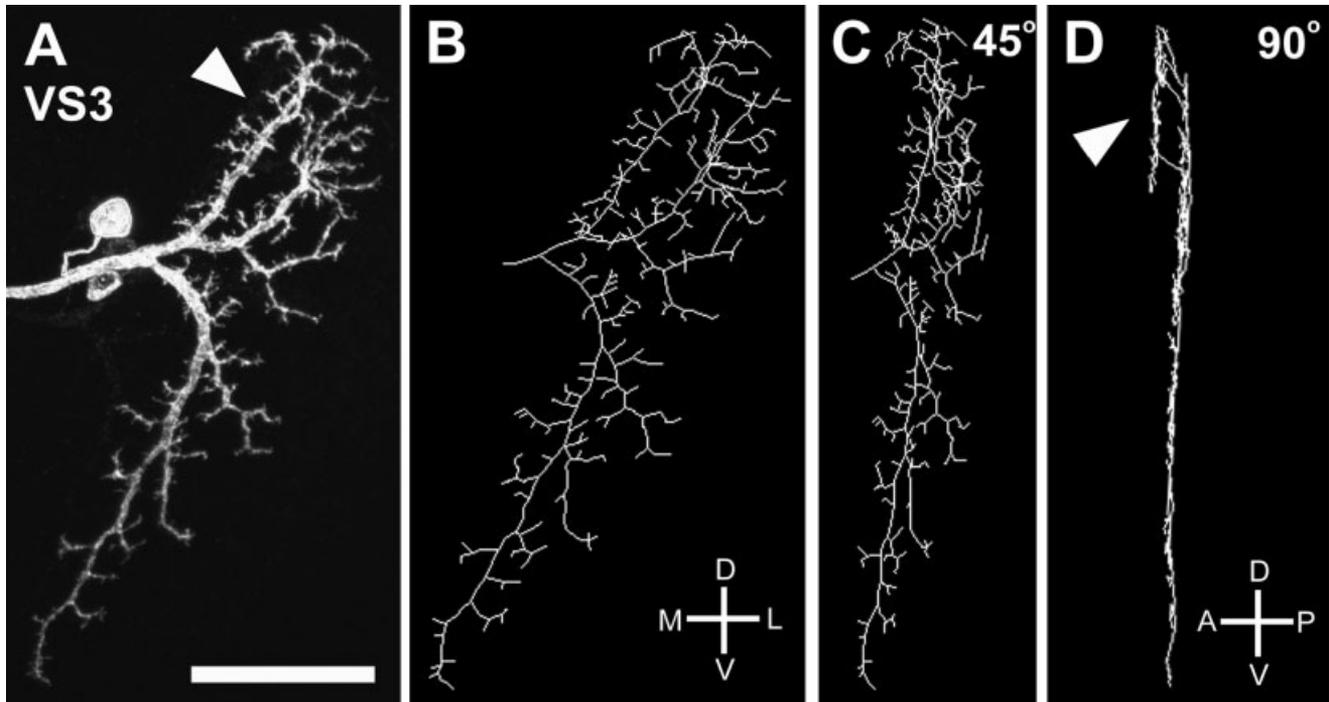
Fig. 6.   The VS3 neuron. **A:** Posterior view of the dendrites of the VS3 neuron. The dendrites are almost symmetrical dorsoventrally and are not as heavily defined by a single main dendritic shaft as those of VS1 and VS2. Note that the major dorsal dendrite continues to extend slightly laterally as it extends dorsally (arrowhead). **B:** Three-dimensional tracings of the dendrites from A, viewed from the same perspective. **C:** The same tracing, rotated 45°. **D:** The same tracing, rotated 90°, showing complex anterior extensions (arrowhead). For details on the structure of this cell, see the text. Scale bar = 25 μm and applies to all panels.

dendrites tend to remain unbranched until they reach a certain anterior-posterior depth, at which point they elaborate in a nearly two-dimensional manner (Fig. 11C–E). Among the cells extending dendrites anteriorly, VS5 appears to innervate most anteriorly, whereas VS1 and VS3 extend dendrites less dramatically in the anterior direction. The VS2, VS4, and VS6 neurons appear to branch mostly or completely posteriorly. This may mean that the cells receive specific types of visual information based on the anterior-posterior depth that their dendrites reach (see Discussion).

DISCUSSION
Lineage of the VS neurons

Using the MARCM system, we have individually labeled the giant neurons of the horizontal system and vertical system in the *Drosophila* lobula plate. All show complex dendritic arborizations through wide areas of the lobula plate and resemble their counterparts in large flies in all major respects.

In addition to labeling individual cells, the MARCM system can be used to study the lineage and birth order of groups of cells. By varying induction times for mitotic recombination and by looking at the content of partial neuroblast clones, we have previously been able to infer the birth orders for distinct groups of mushroom body neurons and for antennal lobe projection neurons (Lee et al., 1999; Jefferis et al., 2001).

These experiments and others have allowed for the cellular characterization of development in various neural tissues. For example, the approximately 2,400 cells of the *Drosophila* mushroom bodies have been shown to be derived from four neuroblasts, which divide throughout development in the manner diagrammed in Figure 2A (Ito and Hotta, 1992). Three morphologically distinct neuron types are among those born from these neuroblasts, and the different types are born during consistent windows during development (Lee et al., 1999). This provides an example of a neural tissue in which numerous and diverse cells are born from a small number of neuroblasts, each of which makes an indistinguishable contribution (Ito et al., 1997a). The projection neurons of the *Drosophila* olfactory system provide a contrasting program, in which a large number of cells with various different structures are derived from three neuroblasts, each of which makes a unique contribution (Jefferis et al., 2001). These two programs have in common the fact that different morphologically distinct neurons are born in an invariant order and during specific and consistent times of development.

Experiments along these lines were uninformative with regard to the order in which the VS neurons are born. Among single cell clones, we found no correlation between the time of recombination (via heatshock from 0 to 7 days after larval hatching) and the member of the vertical system that was labeled. Indeed, only heatshocks at 2 or 3 days after larval hatching produced single cell clones consistently, and both times provided single cell clones rep-
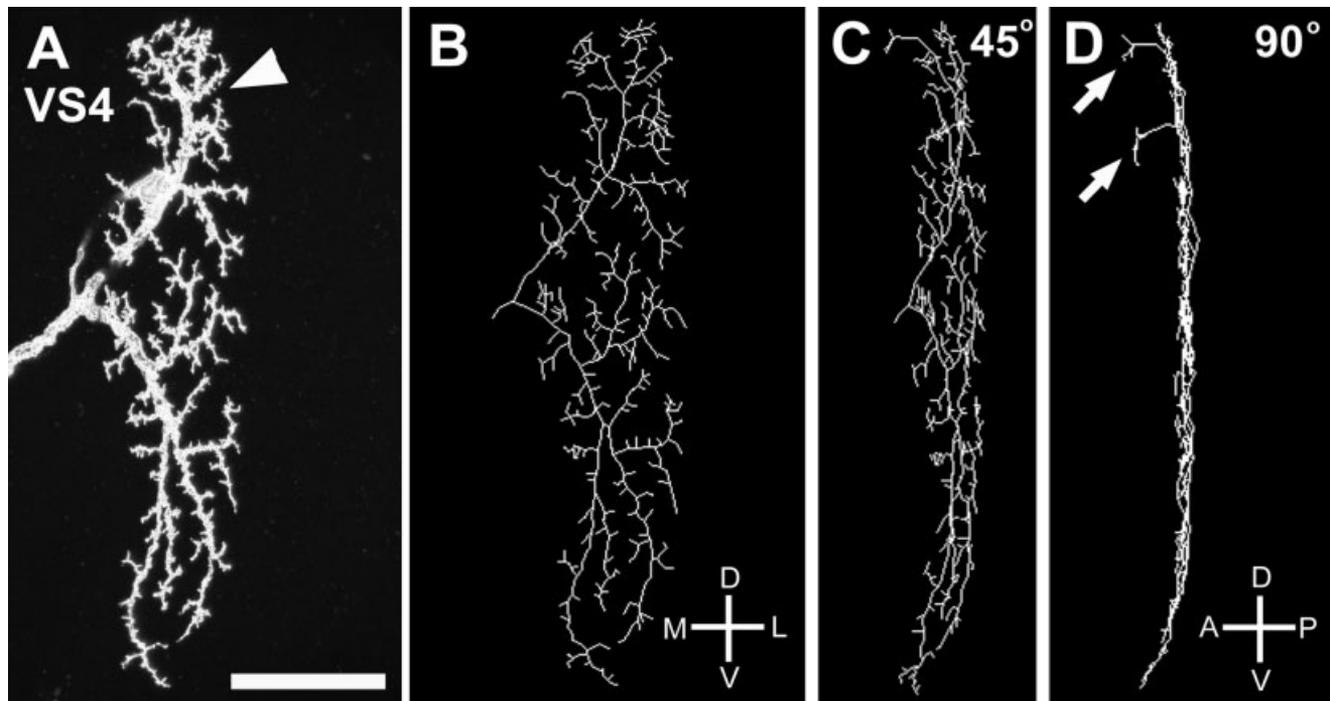
Fig. 7. The VS4 neuron. **A:** Posterior view of the dendrites of the VS4 neuron. Overall, the structure is similar to that described for VS3 (see text and Fig. 6). Unlike VS3, the dorsal dendrite curves medially as it extends dorsally (arrowhead). **B:** Three-dimensional tracings of the dendrites from A, viewed from the same perspective. **C:** The same tracings, rotated 45°. **D:** The same tracings, rotated 90°. Compared with VS3, this dendritic tree shows few and simple anterior extensions among the dorsal dendrites (arrows). For details on the structure of this cell, see text. Scale bar = 25 µm and applies to all panels.

resenting a full range of VS neuron types. This would not be unexpected if the neurons were all born in rapid succession, because there is some heterogeneity in the speed at which different animals develop. We also seldom saw neuroblast clones with fewer than six cells. This, too, is to be expected if the neurons are all born rapidly. With only a narrow window of time between the first and last division of the neuroblast, it would be difficult to create a partial vertical system neuroblast clone. Such a short window is quite possible if only three neuroblast divisions are required for the creation of the vertical system. Combining these results, it is possible that there is no consistent birth order, but it seems likely that the VS neurons are all born in too short a temporal window for them to be distinguished using these methods.

More generally, however, we can use neuroblast clones to infer the lineage of the horizontal and vertical systems. The fact that we see neuroblast clones containing all the VS neurons (Figs. 2, 10) demonstrates that they are related in lineage. The lack of HS cells in vertical system neuroblast clones and the lack of multiple HS cells in the same clone argue that there is no clear lineage shared by the VS and HS neurons, or among the HS neurons. These negative results are, however, less conclusive than the strong positive evidence in favor of lineage within the vertical system. Therefore, as in the mushroom bodies and projection neurons, it seems that the different VS neurons are derived from the same neuroblast, but it is unclear that the cells are born at a precise time in development, or even that there is a particular order in which they are born.

Stereotypy of the VS dendrites

As integrators of information from broad regions of the visual field, VS neurons have dendrites spread over large areas of the lobula plate. Given the retinotopic map present in input to the lobula plate and the separation of motion information into different anterior-posterior layers, it seems likely that the three-dimensional structure of these dendrites is critical to their function. Experiments on dark-reared flies have shown that the function of these cells is not dependent on experience (Karmeiere et al., 2001). This indicates that the inputs to the lobula plate are largely hard-wired and that the dendrites of the VS neurons probably form normally in visually deprived flies. Alternatively, it is possible that the function of these cells might be maintained even if their architecture were slightly perturbed. This possibility was supported by a computer model indicating that variability in the input or specific connections made would probably be tolerated and that the neurons would maintain normal function (Douglass and Strausfeld, 2000a, b).

In an effort to see how consistent the structure of the dendrites is in wild-type animals, we have looked at 12 VS1 neurons from different animals in detail and compared their structures (see Fig. 10 for examples). By observing natural levels of variation, we hope to gauge how strictly the development of these dendrites is regulated. We have found that there is variation in certain branching characteristics but that the overall size and three-dimensional shape of the dendritic field are highly stereotyped. We have also observed that the structure is not
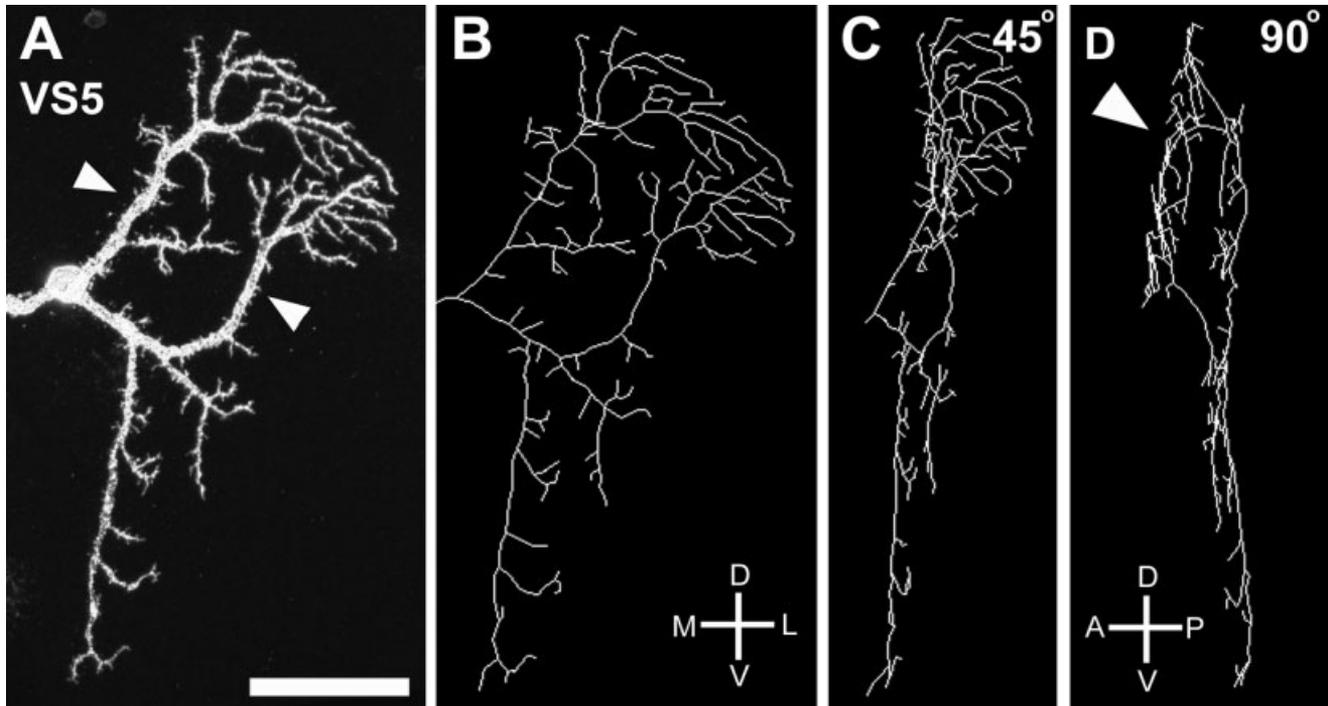
Fig. 8. The VS5 neuron. **A:** Posterior view of the dendrites of a VS5 neuron. The structure is characterized by two separate large branches, which extend dorsally (arrowheads). **B:** Three-dimensional tracing of the dendrites from A, viewed from the same perspective.

C: The same tracing, rotated 45°. **D:** The same tracing, rotated 90°, showing a relatively dramatic anterior extension in the dorsal dendrites (arrowhead). For details on the structure of this cell, see the text. Scale bar = 25 μm and applies to all panels.

changed in any obvious manner in dark-reared flies (unpublished data). This indicates that the region covered by the dendrites of the VS1 cell is consistent but does not answer the question as to whether or not this consistent structure is necessary for the normal function of the cell, nor does it tell whether the input to the structurally normal VS1 cell is perturbed in visually deprived flies.

Similarity to large flies

Our analysis of the *Drosophila* horizontal system has shown that there are three HS neurons that send their dendrites into distinct but overlapping fields in the lobula plate. In these respects, our results are consistent with past studies in *Drosophila* (Fischbach and Dittrich, 1989) and indicate that the horizontal system in *Drosophila* is identical schematically to those described for larger flies (Pierantoni, 1976; Strausfeld, 1976a).

A detailed structural analysis of the *Drosophila* vertical system allows us to make comparisons between it and the systems of large flies. The most noticeable difference between *Drosophila* and large flies is the reduced number of VS neurons. Whereas blowflies and houseflies have 9–11 vertical system cells, we found 6 in *Drosophila*. Because the marking of cells with the MARCM system depends on the expression of a Gal4 driver, it is possible that there are additional members of the *Drosophila* vertical system that lack expression by Gal4-3A. Such cells would not be stained in these experiments. There are a few arguments against this possibility.

First, the VS neurons that we have described all share a common lineage, as shown by their common appearance in neuroblast clones. They also have in common strong Gal4-3A expression (Fig. 1B). It seems unlikely that one or a few additional cells sharing the structural and functional characteristics of VS1-6 would appear in the vertical system without sharing lineage and gene expression with the others. Second, our total of six VS neurons is in accord with a previous estimate of five to seven (Heisenberg et al., 1978), and we have described all the vertical system members identified in this previous study. Since the estimate of five to seven was reached via silver staining, it was not dependent on the expression of Gal4-3A or any other driver. Finally, the cellular structures that we have described here provide a structural counterpart for the vertical systems of larger flies (see below). There are few or no VS neurons in large flies that lack close structural relatives among VS1–6. This provides support for the idea that six *Drosophila* VS neurons would be sufficient to carry out the same function as the blowfly and housefly vertical systems.

Although the *Drosophila* vertical system has fewer cells, the individual neurons bear a striking resemblance to those in the housefly and blowfly. The blowfly, for instance, has 11 VS neurons with characteristic dendritic structures that have been described in detail (Eckert and Bishop, 1978; Hengstenberg et al., 1982; Krapp et al., 1998). VS1 in the blowfly and VS1 in *Drosophila* closely resemble each other, with a ventrally sweeping dendritic shaft that remains at the posterior edge of the lobula plate and an elaborate dorsally projecting dendrite that extends anteriorly. In the blowfly, VS2–6 show a mix of dendritic structures that either sweep ventrally as VS1 does or are
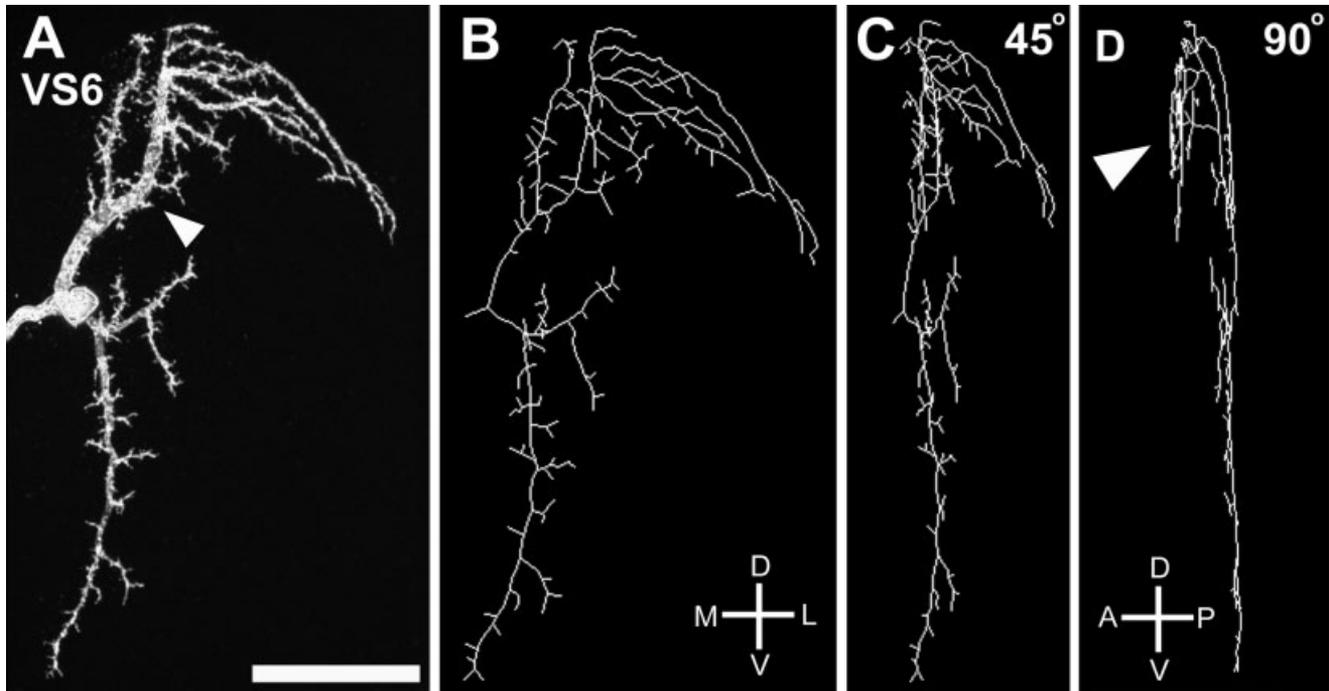
Fig. 9. The VS6 neuron. **A:** Posterior view of the dendrites of a VS6 neuron. Like VS5, VS6 has its dendrites predominantly projecting dorsally. Unlike for VS5, these dorsal dendrites derive from a single major dorsal branch (arrowhead). **B:** Three-dimensional tracing of the dendrites from A, viewed from the same perspective. **C:** The same tracing, rotated 45°. **D:** The same tracing, rotated 90°, shows anterior extension of the dorsal dendrites, but not as dramatically as in VS5 (arrowhead). For details on the structure of this cell, see text. Scale bar = 25 µm and applies to all panels.
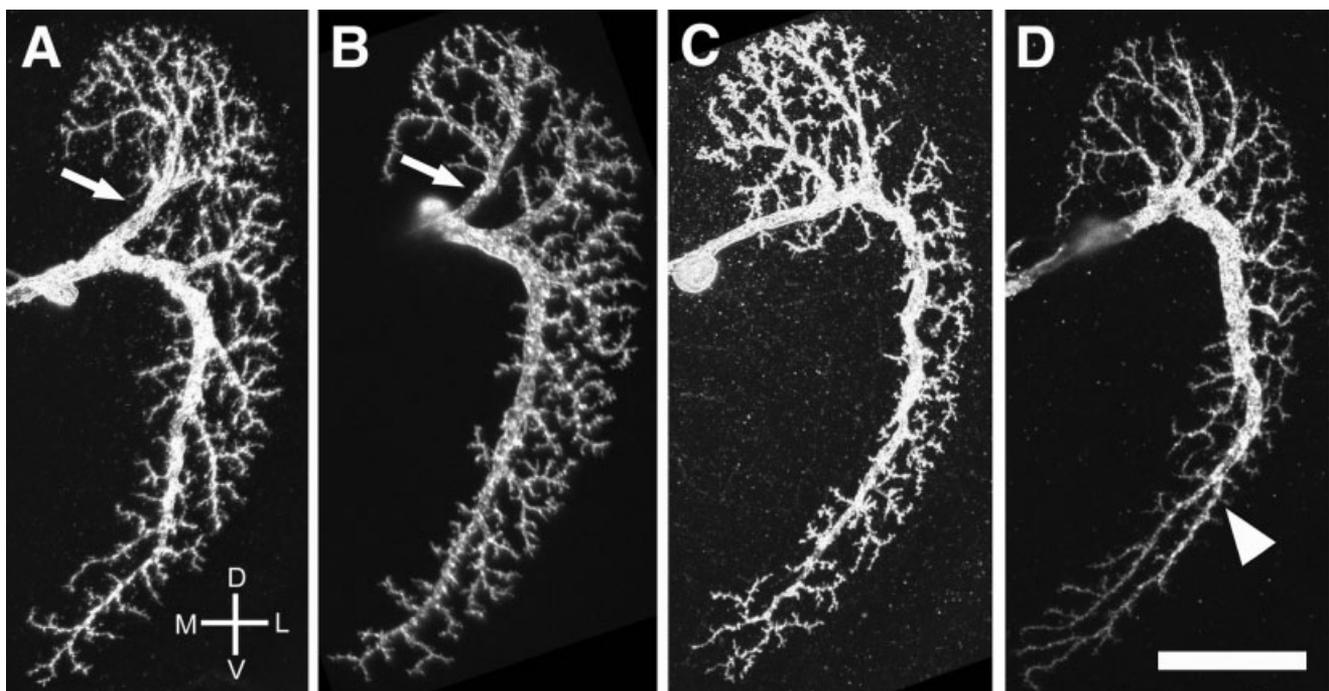

Fig. 10. Stereotypy in the VS1 dendrites. All panels show examples of wild-type VS1 dendrites. **A,B:** Dendritic trees in which single large branches (arrows) account for all of the dorsal dendrites. **C,D:** Dendrites with multiple dorsal branches filling this same space. A–C: Single, unbranched dendritic shafts that extend to the ventral tip of the dendritic tree. D: A cell with a branched dendritic shaft (arrowhead). Scale bar = 25 µm.
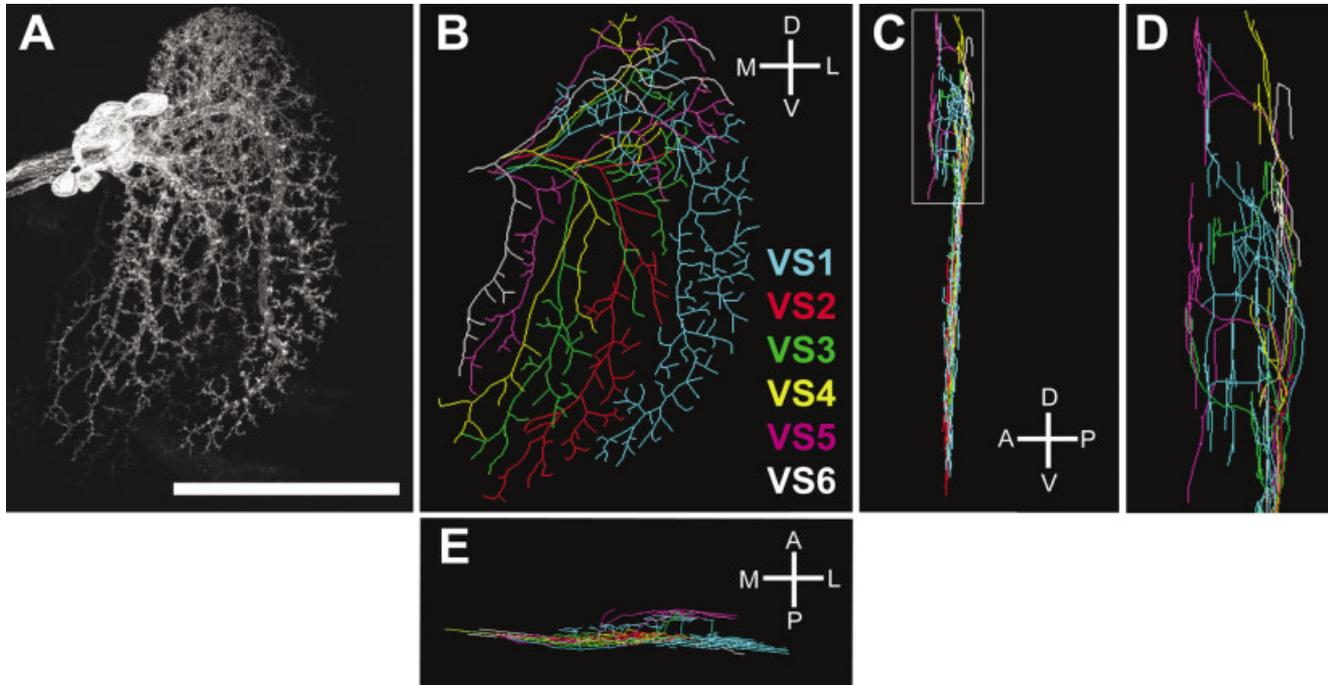
Fig. 11. The three-dimensional structure of a vertical system neuroblast clone **A:** Posterior view of the dendrites of the vertical system. All six cells are visible. **B:** Tracing of the dendrites from A, with each cell's dendrites in a different color. The color for each individual cell is indicated. Due to the difficulty of determining the origin of certain dendrites, some have been left out of the traces. Most notably, the dorsally projecting dendrite of VS2 is absent from the tracing, and major aspects of VS3 and VS4 have been left out. **C:** The same tracing from B, rotated 90° to show anterior-posterior depth. **D:** Closer view of the boxed area in C showing the anterior-posterior details of the dorsal dendrites. **E:** Dorsal view of the tracing from B. Scale bar = 50 μm and applies to all panels except for D.

approximately symmetrical along the dorsal-ventral axis. For the most part, the dendrites of these neurons are on the posterior face of the lobula plate, but some have a few anteriorly extending dendrites in the dorsal lobula plate. These cells in the blowfly correspond structurally to VS2–4 in *Drosophila*. The remainder of the blowfly VS neurons have dendrites similar to VS5 and VS6 in *Drosophila*. In both flies, the ventral dendrites of these neurons show few if any major branches, and the complex dorsal dendrites extend anteriorly.

A recent study in the blowfly linked members of the vertical system with the specific optic flow events to which they responded optimally (Krapp et al., 1998). The conclusion was that different members of the vertical system are most responsive to optic flow events that would accompany different rotational body movements during flight. As a group, the vertical system neurons combine to be sensitive to rotations around various body axes, ranging from pure pitch changes to pure roll. Generally, it was found that the three-dimensional structures of given cells' dendrites correspond to the part of the visual field sensed and the type of motion to which the cell is optimally responsive (Krapp et al., 1998). Of four discrete layers of input into the *Drosophila* lobula plate, the anterior two contain horizontal flow information whereas the posterior two layers contain vertical flow information (Buchner and Buchner, 1984). The similarities in dendritic structure between blowflies and *Drosophila* and the nature of the input into the lobula plate support the idea that the *Drosophila* VS neurons may respond to pitch and roll stimuli

similar to those sensed by their counterparts in large flies. Of course, the current evidence is indirect, and functional studies similar to those done in blowfly (Krapp et al., 1998) would be necessary to confirm these functions for the dendritic structures described here for *Drosophila*.

The clear positive staining of these cells in a dark background leads us to believe that the vertical system will serve as a system in which *Drosophila* genetics can be employed to study the function and importance of these cells for flight control or other visually dependent behaviors. For instance, Gal4-3A could be used in conjunction with a UAS transgene that interferes with changes in membrane potential (reviewed by White et al., 2001) or a UAS-driven apoptotic gene such as *hid* to kill developing VS neurons (Grether et al., 1995). However, more vertical system-specific GAL4 drivers may be necessary for these experiments to be most effective. In addition to its potential usefulness in studies of visual behavior, the VS neurons could be used as a model for studying dendrites in general. Through use of the MARCM system, loss of function alleles in genes of interest could be made homozygous in individual VS neurons, and the effects on their complex yet stereotyped dendritic structures could be studied.

ACKNOWLEDGMENTS

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