Computer Reconstruction of All the Neurons in the Optic Ganglion of Daphnia magna

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ABSTRACT
The cellular architecture of the Daphnia compound eye visual system was studied by using computer-aided techniques. All the neurons in one half of the bilaterally symmetric optic ganglion (OG) were reconstructed in three dimensions from serial electron micrographs. The techniques employed were those developed by Levinthal and collaborators (Macagno, Levinthal, and Sobel, Ann. Rev. Biophys. Bioeng. 8:323-351, 1979). The approximately 200 neurons reconstructed were classified according to where they branch in the OG (the lamina and/or the medulla) and whether they send processes to the supraesophageal ganglion and/or across the midplane. Within each class, neurons were further characterized according to cell body location and size and location of their branching fields. Centrifugal processes from neurons with cell bodies not in the OG were also identified. These results provide the bases for a detailed examination of the synaptic connectivity of the identified neurons and for hypotheses concerning their functional roles in visually evoked behaviors.

Key words: visual system, neuroanatomy, crustacea, 3-D reconstruction

There are several important reasons to undertake a detailed and complete anatomical reconstruction of a neuronal network at the cellular and synaptic levels. One is that the data thus obtained (e.g., the cell body location, branching pattern, and distribution of synaptic sites and other organelles of each neuron, and the interrelations and patterns of synaptic connections among neurons of the network) can play a significant role in the formulation of hypotheses concerning the functions of each element within the network and of the network as a whole. These hypotheses can then be tested by using either intracellular recording and dye-marking techniques to study selected anatomically identified neurons or voltage-sensitive dyes to record the activity of a subpopulation of these neurons (Salzberg et al., '77). Another reason is that detailed information about the structure of the end product reveals the requirements which are placed upon the mechanisms responsible for the assembly of the network during neurogenesis (Macagno et al., '73; LoPresti et al., '73; Ward et al., '75; Ware et al., '75). Furthermore, detailed information of this kind is very useful in interpreting the effects of experimental perturbations of the developmental process, such as the deletion of individual neurons or groups of neurons by genetic or other means. It is largely for these reasons that we have undertaken the computer-aided three-dimensional (3-D) reconstruction from serial electron micrographs of all of the approximately 200 neurons in one half of the bilaterally symmetric adult optic ganglion of the small crustacean Daphnia magna. An additional motivation for undertaking this rather arduous task is provided by recent progress (summarized below) in our understanding of the development and of the physiological and behavioral attributes of this visual system. We present here our observations on the location and branching patterns of these neurons; their synaptic connections will be the subject of future reports.

The compound eye visual system of Daphnia has been the subject of numerous anatomical studies with the light microscope (Retzius, '06; Leder, '15; Aramant and Elofsson, '76; Nässel et al., '78) and the electron microscope (Röhl and Törö, '65; Bohn and Parker, '68; Wolff and Güldner, '70; Güldner and Wolff, '70; Macagno et al., '73; Macagno and Levinthal, '75; Sims and Macagno, '83). It consists principally of a single, medially located compound eye, and a single, also medially located, optic ganglion (OG). (A schematic drawing of the visual system is shown in Fig. 1a.) In addition, various regions of the supraesophageal ganglion

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(SEG) are innervated by neuronal processes of cells of the OG. The SEG also contains five pairs of motoneurons which connect to the three pairs of muscles that move the eye (Consi and Macagno, '82).

The OG is particularly appropriate for complete reconstruction. For one thing, it contains a relatively small number of neurons (about 400). Because of bilateral symmetry, only half of these neurons need to be reconstructed. Furthermore, it is physically small (about 100 \( \mu \)m \( \times 70 \mu \)m \( \times 120 \mu \)m). This means that a complete hemiganglion is contained in about 1,200 serial electron micrographs shot at a magnification of about 700, which is adequate for following most neuronal processes from micrograph to micrograph and for recognizing sites of synaptic contact.

In addition to the anatomical studies of the adult visual system of Daphnia mentioned above, the mechanisms involved in its development have been extensively studied in our laboratory (reviewed in Flaster et al., '82). Furthermore, investigations of the oculomotor responses of Daphnia to photostimulation (Frost, '75; Young, '74, '77; Downing and Young, '75; Young and Downing, '76; Ringelberg et al., '75; Buchanan and Goldberg, '81; Consi and Macagno, '84), studies of polarization sensitivity (reviewed by Waterman, '81), and the recent determination of the spectral sensitivity of the photoreceptors in the compound eye (Schehr and Young, '75; Buchanan and Goldberg, '81; Consi and Macagno, '84) provide important information about the functional attributes of the system. Understanding the results of these studies in terms of the cellular substrate involved also requires the detailed structural information about the optic ganglion of Daphnia which we begin reporting below.

METHODS

The animals used in this study were large adults taken from a laboratory culture of Daphnia magna which we have maintained for a number of years. The growth conditions are such that reproduction is exclusively by parthenogenesis.

The tissue was prepared for electron microscopy as previously described (Macagno et al., '73). In brief, Daphnia were fixed by immersion in buffered osmium tetroxide, stained en bloc with uranyl acetate, and embedded in epon. Serial 0.1 \( \mu \)m sections of the entire optic ganglion (about 1,200 sections) were cut and post-stained with lead citrate. The sections were then photographed on Kodak microfilm at 685 x on a Zeiss EM9 S electron microscope.

The electron micrographs were sequentially aligned and copied onto a 35mm filmstrip, one section to one frame, using an Image Combiner (Macagno et al., '79). To enter data into the computer, the filmstrip is back-projected onto a ground glass screen and viewed in superposition with a computer graphics display screen (Levinthal et al., '74). The perimeter of any object in a given electron micrograph (e.g., a neuronal process) is then reproduced ("traced") on the computer screen using a digitizing tablet and a pointer. (This is called "contour" mode.) The outline of the same object is again traced as it appears in subsequent frames of the filmstrip (i.e., subsequent sections through the ganglion). Computer programs (the CARTOS system for Computer Aided Reconstruction by Tracing of Serial Sections; Macagno et al., 1979) combine the 2-D information traced in each frame with the frame number and the thickness of the tissue slices to produce the 3-D computer reconstruction of the object. Alternatively, the branching pattern of a neuron can be recorded by marking a point at the center of a particular neuronal process in each of the frames through which it is traced, producing a sequence of connected vectors along the axis of each process of the neuron. (This is called "tree" mode. The neuropils, the neuronal cell bodies, and the processes of a few neurons were traced in contour mode; the processes of most neurons were traced in tree mode.

At the magnifications used in this work, slightly more than one half of the optic ganglion was included within each frame of the filmstrips employed in the computer reconstructions (see Fig. 1a). All the neurons in one such filmstrip were reconstructed, as well as a few neurons in filmstrips of other specimens. To check for consistency and reproducibility, about 10% of the neurons were reconstructed by both investigators. No significant differences were found in the duplicated reconstructions.

The low magnification used, the distortions due to shrinkage of sections under the electron beam, the thickness of the sections (90-100 nm), and the occasional loss of sections in the series all contribute to difficulties in following every process of each neuron from section to section in the filmstrip. This is particularly true for the very fine neurites the diameters of which are of the same magnitude as the section thickness. The reconstructions of neurons presented here thus include all their major branches as well as most of those with diameters at least 0.2-0.3 \( \mu \)m, but generally lack the very fine processes which are just visible in Golgi (Nässel et al., '78) or HRP-filled (Schehr, personal communication) preparations. In previous reconstructions, of smaller volumes and carried out at higher magnifications, we were able to reconstruct many of the fine processes belonging to photoreceptor terminals and their target neurons in the OG (Macagno et al., '73). We found no synaptic sites on the finest processes, which leads us to believe that the reconstructions discussed in this report include most of the functionally interesting parts of the neurons.

RESULTS

The computer reconstructions presented below are those carried out, for the most part, on a single serially-sectioned specimen, in which all the neurons with cell bodies in the left optic hemiganglion were reconstructed. All centrifugal processes entering the optic ganglion from the suprasexual ganglion were also reconstructed. Comparison with other specimens (serially or non-serially sectioned) reveals that the anatomical features described here, and particularly all the classes of neurons, are common to the animals in our Daphnia clone. Many of the reconstructed neurons were also identified in other serially-sectioned specimens, but only a few of these were reconstructed. In these cases, a close similarity but not an identity of branching pattern was observed among the reconstructions of a cell in different specimens, in accord with a previous report (Macagno et al., '75).

General features of the optic ganglion

The optic ganglion consists of two regions, the lamina and the medulla. The lamina is the more anterior of the two and the one to which the photoreceptor axons project. Its bilaterally symmetric neuropil is continuous between left and right (see Fig. 1a.b). The medulla is the more posterior region and it connects to the suprasexual ganglion. It consists of separate left and right lobes, each with its own neuropil (see Fig. 1a.d). The medullary neuropils are separated from the laminar neuropil by a region lacking fine
Fig. 1. a. Schematic diagram of the *Daphnia* compound eye visual system. Dorsal view; anterior is up, posterior down. CE, compound eye; RA, photoreceptor axons; L, lamina; M, medulla; S, supraesophageal ganglion. The laminar and medullary neuropils are indicated within the optic ganglion. The shaded area, the left side and part of the right side of the optic ganglion, was the region reconstructed from serial electron micrographs of sections cut perpendicular to the antero-posterior axis; b, c, and d indicate the level of three such sections shown in the other panels of this figure. b-d. Electron micrographs of sections of one specimen at the levels indicated in a. The arrowheads indicate the position of the midline; ventral is up. The section shown in b is in the lamina; the section in c is at the boundary between lamina and medulla; the section in d is in the medulla. Open arrows point to some of the 11 glial nuclei found in a hemiganglion. The letters (a through k) with small arrowheads point out the principal process of one LMc neuron (see text) from each of the 11 optic cartridges on the left side of the optic ganglion to demonstrate that the relative positions of optic cartridges remain the same throughout the ganglion. The bar in d = 10 μm.

The morphological bases for the classification of neurons

Various schemes have been used to classify and name visual interneurons of arthropods, relying for the most part on the location of cell bodies or the pattern and location of their branches (Strausfeld and Nässe, '81). Neurons with cell bodies in the *Daphnia* OG may branch in the lamina, or in the medulla, or in both, or in neither. In addition, they may send a process across the midplane, to the opposite side of the ganglion, or they may send a process posteriorly, out of the OG and into the supraesophageal ganglion (SEG). Since it is reasonable to assume that different types of
analysis of visual information are carried out in each region (Laughlin, '81), we chose to classify the ganglion neurons according to where they branch. We used L and M to denote ipsilateral branching in the laminar and medullary neuropils, and B (for bilateral) and S to denote the sending of processes to the opposite side of the OG and to the SEG, respectively. Of the 15 classes of neurons with cell bodies in the OG which are logically possible with this nomenclature, 11 were found among the reconstructed cells. Three classes (L, M, LM) branch only in one side of the OG; four classes (LS, MS, LMS, S) send processes to the SEG; three classes (LB, MB, LMB) send major processes across the midplane of the OG. Only one cell was found that sends major processes to both the other side and to the SEG (an LSB cell).

Among those neurons which branch in both the laminar and medullary neuropils (the LM class), those with cell bodies at the anterior surface of the OG were placed into a subclass, the LMc cells (the c stands for columnar, a geometrical characteristic of this class) because of the important role played by these cells in the organization of the ganglion, as described below. Two other elements were found in the OG: terminals of photoreceptor axons (the R class), and centrifugal processes of SEG neurons (the CS class). The corresponding cell bodies are not found within the OG, but in the compound eye and the SEG, respectively. The numbers of neurons within each of these classes found in the hemiganglion in which all the cells were reconstructed are listed in Table 1. Their cell bodies are displayed in Figure 2. (With respect to the S class, since the exact boundary between the OG and the SEG is not well-defined by, for example, a region devoid of cell bodies, the number of S neurons counted in this specimen is not exact. Had we included a few more serial sections in the filmstrip, we would have counted more than 11 S neurons. The significant observation is that at least some of the S class is present in the OG.)

Computer reconstructions of neurons in the classes defined above are shown in Figures 3 through 12. Since the number of cells in some of the bilateral classes is small, and since they cross the midplane together at the commissures, the LB, MB, and LMB cells have been displayed together in Figures 7 and 8. The LSB cell is much more similar to the LS cell than to the other bilateral cells and does not cross the midplane at the commissures. It has therefore been displayed with the LS cells. Because of space limitations, all of the over 200 computer reconstructions can not be reproduced individually here (they are available to the interested reader, however). Instead, the figures present, for each class, the branches of all the reconstructed neurons in that class and some examples of individual neurons. Although displaying all the branches together yields rather complicated figures, these are included because they do show how each class occupies the space of each neuropil.

Within each class, various characteristics of the individual neurons were examined. These include the location and the diameter of the cell body; whether one or more processes emanate from the cell body (monopolar, bipolar, multipolar); whether branches are restricted to a small region or extend widely within a neuropil (small-field, large-field); and whether branches are restricted to particular levels or regions of the neuropils. These characteristics are described in the sections that follow. Numerical data are presented in Table 1. The photoreceptor axon terminals and the LMc neurons will be described in detail first, as they provide the geometric and retinotopic frameworks for the ganglion.

The photoreceptor axon terminals

The eight photoreceptor axons from each ommatidium enter the laminar neuropil through its anterior surface and, together with the main processes of five neurons (the LMc cells described in the next section), travel posteriorly as a group, forming a column known as an optic cartridge. Each side of the optic ganglion contains 11 such columns, travelling from anterior to posterior through the lamina and medulla. These columns correspond to the 11 ommatidia on the same side of the compound eye (Macagno et al., '73). All photoreceptor axons terminate within the laminar neuropil, but the LMc neurons continue into the medullary neuropil. The computer reconstructions of the eight photoreceptor axons from one optic cartridge are shown in Figure 3a. The terminals have been spread out laterally so they can be visualized individually. The larger branches of the terminals remain within the column, whereas some of the very fine processes (not shown) can extend into adjacent columns.

Within each column, individual photoreceptor axons branch and terminate in a stereotyped manner. For example, there are two photoreceptors in each ommatidium, numbers 2 and 6 (see Flaster et al., '82), whose rhabdomeric microvilli are oriented dorso-ventrally (parallel to the animal's midplane and perpendicular to the microvilli of the other six photoreceptors). Their axons (R2 and R6 in Fig. 3a) always branch and terminate first, in the anterior third of each column. Three of the remaining six axons (R1, R3, and R5) branch almost entirely in the anterior two thirds, the other three (R4, R7, and R8) in the posterior third of each column. These branching patterns, repeated from column to column, define three strata in the laminar neuropil, Is-i (anterior third), Is-ii (middle third), and Is-iii (posterior third), with respect to which the branching patterns of ganglionic interneurons can be examined (see Fig. 3a).

Laminar-medullary-columnar (LMc) neurons

Among the ganglionic interneurons which branch in both the laminar and medullary neuropils, the LMc neurons

| Table 1. Summary of the Characteristics of the Various Cell Classes Found in the Reconstructed Left Half of the Optic Ganglion of One Specimen |
|-----------------|----------------|----------------|----------------|
| Class          | No. of cells | Cell body diameter | No. mono- | No. multi- | No. small- | No. large- |
| L              | 44           | 6.0 ± 0.6          | 7          | 0          | 2          | 5          |
| M              | 17           | 5.5 ± 0.6          | 5          | 0          | 12         | 3          | 4          |
| LM             | 27           | 6.2 ± 0.6          | 13         | 0          | 14         | 10         | 17         |
| LMc            | 55           | 6.7 ± 0.5          | 44         | 11         | 55         | 0          | 0          |
| R             | 17           | 7.5 ± 1.1          | 17         | 0          | 9          | 8          |
| LS             | 17           | 6.8 ± 1.2          | 2          | 10         | 18         | 7          |
| MS             | 36           | 6.4 ± 1.2          | 6          | 30         | 28         | 8          |
| LMS            | 17           | 6.5 ± 0.9          | 3          | 14         | 7          | 10         |
| S             | (11)         | 4.9 ± 0.3          |             |            |             |            |
| CS             | (11)         | 6.3 ± 0.3          |             |            |             |            |
| Totals         | 193          | 6.5 ± 1.2          | 97         | 96         | 134        | 59         |

1Includes bipolar neurons.
2Small-field neurons branch within three adjacent columns, large-field in more than three or in non-adjacent columns.
3Includes 12 LS, 1 MB, and 4 LMB bilateral neurons.
4Includes the single LSB neuron.
5This number is not significant, since it is determined solely by the point where the reconstructions are stopped in the region between SEG and OG (see text). It is not included in the total.
6Cell bodies not in the OG and presumed to be in the SEG. Not included in the totals.
Figure 2
Fig. 2. Locations of the cell bodies of the neurons in each of the cell classes in the left half of the optic ganglion. Two views are shown for each cell type. The orientations are indicated by the small squares at lower right in a, b, and d (L, lateral; M, medial; V, ventral; D, dorsal; A, anterior; P, posterior). The left half of the laminar and the left medullary neuropils (indicated by LN and MN in panel a) are shown for positional reference. a,b. LMc cells, dorsal and anterior views. c,d. L cells, dorsal and posterior views. These same two views are shown for the rest of the cells. e,f. M cells. g,h. Bilateral cells (including LB, MB, and LMB cells). i,j. LS cells (including the LSB cell). k,l. MS cells. m,n. LMS cells. o,p. S cells. Orienta-
tion squares are 10 μm on a side.

have their cell bodies on the anterior surface of the ganglion (see Fig. 2a), separate from the rest of the cell bodies of the optic ganglion.

The LMc cells each have a single main process which extends posteriorly through the laminar neuropil, in parallel with the photoreceptor axons, and beyond them into the medullary neuropil (Fig. 1). As mentioned in the previous section, five LMc cells associate and form an optic cartridge or column with each bundle of photoreceptor axons in the laminar neuropil. The groups of five LMc cells continue to describe separate columns in the medullary neuropil, though some overlapping or mixing among the groups occurs in the extreme posterior region of the medullary neuropil. An example of one such group is shown in Figure 3b.

Five LMc cell subtypes, each found once in every optic cartridge, can be identified on the basis of the position of the cell body, the antero-posterior location of the branches extending from the main process, and synaptic connections with the photoreceptors (Macagno et al., '73). These subtypes are referred to as LMc1 through LMc5 (labeled L1 through L5 in Fig. 3b). For example, the LMc2 cells are characterized by having two regions of branching within the laminar neuropil (one in Is-i and the other in Is-iii), by having somewhat larger cell bodies than the other four LMc subtypes (about 8 μm diameter versus 6.5 μm) and by receiving synaptic inputs from photoreceptors 2 and 4 (see Fig. 3b). Furthermore, the main processes of the LMc2 and LMc3 cells in each column run in contact with each other throughout most of the laminar neuropil, so that a horizontal section through any level shows the main process of an LMc3 cell flattened against the larger main process of an LMc2 cell. The relative positions of the five LMc cells in a column are constant among ipsilateral columns and mirror symmetric with those of contralateral columns. These relationships have been found in all of the several specimens examined. Previously published results (Macagno et al., '73) and other results of this work which will appear subsequently provide further details of this subtyping and of the synaptic connectivity between photoreceptors and LMc cells.

The subdivision of the ganglionic neuropil into 11 columns permits us to classify cells according to field size. A cell in the optic ganglion which branches in three or less adjacent columns is arbitrarily termed small-field, and a cell which branches in more widely spaced columns is termed large-field.

**Morphological characteristics of other elements of the optic ganglion**

**Cell body size and location.** The cell bodies of neurons in each class are, for the most part, clustered in discrete regions of the ganglion (see Fig. 2). However, with the exclusion of the LMc neurons, cell bodies of different classes
Fig. 3. Photoreceptor axons and terminals and LMc neurons of a single midline optic cartridge (the "b" cartridge; see Fig. 1b), shown in a medial view, anterior up. a. The axons and terminals (R1-R8) of the eight photoreceptors. The computer reconstructions, which have been separated for clarity, begin near the anterior surface, as the optic axons enter the ganglion. R2 and R6 terminate most anteriorly in all cartridges examined, R4 and R7 most posteriorly, close to the lamina-medulla boundary. Thus, the receptor terminals subdivide the laminar neuropil into three strata, Is-i, Is-ii, and Is-iii, as indicated on the right side of the figure. b. The five LMc cells (L1-L5) of the optic cartridge, along with three of the photoreceptors shown in a. The nuclei have also been displayed in the cell bodies, which lie close to the anterior surface of the ganglion. The three photoreceptor axons have been placed beside the LMc cells with which they make synapses. Thus R2 makes synapses onto L1 and L2, R4 onto L2 and L3, and R5 onto L4 (L5 receives synaptic inputs from R1). The arrowheads indicate the level of the lamina-medulla boundary. Note that no photoreceptor axon enters the medullary neuropil, but that all five LMc cells do. Bars = 10 μm.

overlap to some degree with those of other classes. Since there is little difference in the cell body size among these classes (see Table 1), it is difficult if not impossible to identify a neuron as belonging to a specific class purely by examining its cell body. However, cell body location does narrow down the possibilities to two or three classes.

**Number of processes emanating from the cell body.** About half of the neurons found in the OG are monopolar; the rest are either bipolar or multipolar (see Table 1). Among the LMc neurons, one of the subclasses (LMC2) has one large process as well as a few smaller ones leaving from the cell body (see cell L2 in Fig. 3b) while all the others are monopolar. Among the other classes, only the L (Fig. 4) and the bilateral (Figs. 7,8) are exclusively monopolar; the others include the different types of geometries. For example, many of the M neurons (Fig. 5) have more than
two neurites emanating from the cell body. In the LM class, 14 neurons are truly bipolar (see Figs. 6c,f), sending two major processes from the cell body, one anteriorly to the laminar neuropil and a second to the medullary neuropil. Among the 13 monopolar LM neurons, most have a single short process that bifurcates, one branch going to each neuropil. A few of the LM neurons with very posterior cell bodies, however, send a single process which branches in each neuropil (Fig. 6e).

Extent and location of branching fields. Every class, with the exception of the LMc neurons, contains both small-field and large-field cells (see Table 1). As defined above in the section on LMc neurons, small-field neurons are those whose branches extend into three or fewer adjacent columns in the neuropil. To the extent that the columns represent retinotopic information, we can conjecture that the small-field neurons in each class conserve such information. Examples of small-field neurons in various classes appear in Figures 5c,d, 6e,f, and 9e,f. Among large-field neurons, some branch widely and some in more restricted areas. Two examples of the latter appear in Figures 9c,d and 10c,d. Among the LM neurons, 26 of the 27 branch in both laminar and medullary regions of the same column in at least one column (see, for example, Fig. 6f).
Among the 18 neurons which send a major process across the midplane, 14 follow the ventral commissure and three follow the dorsal commissure (see Fig. 8). The other one, the single LSB cell, crosses elsewhere (see Fig. 9c,d). Seven other neurons also send processes across to the other side of the ganglion, but these processes are small and secondary, and do not extend far from the midplane. They cross individually and not as part of the commissural bundles.

All the neurons in a class can be displayed together in order to ascertain whether a class branches in a particular region of a neuropil, or whether different classes occupy separate or overlapping regions of the ganglion. Furthermore, because of the retinotopic organization of the OG, innervation of only certain areas may signify that a class is predominantly concerned with information from a specific region of the visual world. When neurons are thus examined together, variations in the extent to which a neuropil is innervated by a class are apparent. For example, the L neurons branch for the most part only in the dorsal half of the laminar neuropil (see Fig. 4a,b). In an-
Figure 6
The bilateral neurons, including the LB, MB, and LMB cells (but not the LSB neuron, which is displayed with the LS cells). a,b. Lateral and anterior views of the whole group (17 neurons). Some of the cell bodies are not shown. The ventral commissure (large arrowheads) and the dorsal commissure (small arrowheads) are indicated in b. The arrows labeled 8a and 8b in a indicate the levels of the micrographs shown in Figures 8a and 8b, respectively. c,d. Lateral and anterior views of neuron m40.

In those cases where the same cell was reconstructed in both sides, mirror symmetry was readily apparent (e.g., Fig. 8c).

DISCUSSION

The computer reconstructions of the optic ganglion of *Daphnia* presented in the previous sections provide us with information about the overall organization of the ganglion and about the types of neurons it contains and their distributions.

The columnar organization of the optic ganglion

Each half of the optic ganglion is organized into 11 parallel antero-posterior columns. Within the laminar neuropil each column contains the major branches of eight photoreceptor terminals and five LMC ganglionic neurons; the for-
Fig. 8. The commissures and a pair of LB neurons. a, b. Electron micrographs of the sections of the optic ganglion at the two levels indicated in panel a of Figure 7. In both panels the arrowheads at top and bottom indicate the position of the midplane of the optic ganglion, and dorsal is up. In panel a processes crossing the midplane at the ventral commissure are indicated by arrowheads, whereas in panel b processes in the dorsal commissure are similarly indicated. Bar = 10 μm. c. Bilaterally symmetric pair of LB neurons (m1) traced in a second specimen. These neurons cross along the ventral commissure. This is a posterior view, with dorsal up; the midplane has been drawn in and is viewed on edge where indicated by the arrowheads. Bar = 10 μm.

In certain areas of Daphnia neuropil the columnar domains are hard to see in individual sections and can only be identified by tracking the LMc cell processes. In other areas, however, the columnar boundaries are very clearly outlined by the processes of other ganglionic cells, such as the LM cells (see Fig. 6d) or the bilateral cells (see Fig. 7b), which travel horizontally between columns, across the ganglion.
Fig. 9. The LS neurons, including the LSB cell. a,b. Lateral and dorsal views of the entire group (17 neurons), with some of the cell bodies removed. The arrowheads in a indicate the processes exiting from the optic ganglion and entering the supraesophageal ganglion. The arrowheads in b indicate some processes crossing the midplane, but not through the commissures (see Figs. 7,8 and text). c,d. Dorsal and anterior views of the bipolar, large-field LSB neuron (m15). This cell was traced in contour mode (see Methods). The cell body is indicated in c by an arrowhead. This cell forms synapses almost exclusively with dorsal optic cartridge photoreceptor terminals. e,f. Dorsal and anterior views of a bipolar, small-field LS neuron (m82). This neuron branches only at the lateral margin of the laminar neuropil.
Fig. 10. The MS neurons. a,b. Lateral and dorsal views of the entire group (36 neurons) with some cell bodies removed for clarity. Processes travelling to the supraneophagal ganglion exit the optic ganglion posteriorly. c,d. Lateral and dorsal views of a large-field MS neuron (m76). e,f. Lateral and dorsal views of a bipolar, small-field MS neuron (m92).
Fig. 11. The LMS neurons. a,b. Lateral and dorsal views of the whole group (17 neurons) minus some cell bodies. c,d. Lateral and dorsal views of a LMS neuron (m33) which innervates topographically similar regions of the laminar and medullary neuropils. e,f. Lateral and dorsal views of a LMS neuron (m115) which innervates regions in the two neuropils (lateral in the lamina, mostly medial in the medullary neuropil) that are for the most part topographically distinct.
In any case, the visualization of the columnar organization of the ganglion is greatly facilitated by the 3-D computer reconstructions, and such organization is without a doubt a feature of the *Daphnia* optic ganglion, as it is in other arthropods.

The columnar organization of the optic neuropils in *Daphnia* provides a topographical representation of visual information as signals from each ommatidium are channeled centrally in parallel by the LMc neurons. In higher arthropods, such as decapod crustacea and insects, a topographic columnar organization is preserved at more central levels than the first medullary neuropil (e.g., in the second medulla, the lobula, the optic foci, etc; see Strausfeld and Nässel, '81, for a review). We have examined the ganglionic neurons in the *Daphnia* OG which project centrally (the LS, MS, and LMS neurons) to determine whether a topographically ordered projection to higher centers exists in this much smaller arthropod. We have not found such ordering in the OG-SEG connective or in the regions of the SEG we have examined. Whether this signifies that structures analogous to the higher visual centers (e.g., the lobula) are absent in *Daphnia* can not be ascertained from our data at this time. However, the fact that the *Daphnia* visual system is capable of mediating complex visual functions (Frost, '75), such as tracking a contrast edge, which are thought to be mediated by the higher centers in larger
arthropods, implies that such structures or their functional equivalents must also be present in *Daphnia.*

**Stratification of the neuropils**

In addition to subdividing the laminar neuropil into columns, the photoreceptor axon terminals give rise to its stratification, as shown in Figure 3a. In each column, the photoreceptors R2 and R6 from the corresponding ommatidium terminate in the superficial one third of the laminar neuropil, defining the laminar stratum ls-i. Other photoreceptors branch and terminate in the middle and posterior thirds, defining the laminar strata ls-ii and ls-iii.

Our present knowledge of the structural and functional properties of the *Daphnia* compound eye photoreceptors, although incomplete, permits us to propose some functional attributes of the laminar neuropil strata. In each ommatidium, photoreceptors R2 and R6 have microvilli oriented in one direction (parallel to the midplane of the eye) and the six others in an orthogonal direction. Since microvillar attributes of the laminar neuropil strata. In each ommatidium, it would appear that layer ls-i receives information about one axis of light polarization from R2 and R6, whereas ls-ii and ls-iii receive information about the orthogonal axis from the six other photoreceptors in each ommatidium.

With respect to wavelength information, recent intracellular measurements (Schehr and Macagno, '83; Schehr, '84) indicate that the photoreceptors in each ommatidium fall into at least three classes according to wavelength of maximum sensitivity. R1 is most sensitive around 590 nm; R2, R3, and R5 are most sensitive around 510 nm; and R6 and R8 respond maximally at wave lengths around 450 nm. No recordings from R4 or R7 have been made thus far. Since R2 and R6 terminate in ls-i, this layer receives middle and short wavelength information. R1, R3, and R5 branch for the most part in ls-ii, which therefore receives both long and middle wavelength information. Of the three photoreceptors which branch almost exclusively in ls-iii, only R8 has been recorded from and it is in the short wavelength class. Whether this apparent differentiation of laminar strata is functionally significant requires consideration of the synaptic connections made by the photoreceptors, the LMc neurons, and the other neurons in the optic ganglion, and will be the subject of a subsequent report.

Stratification of the medullary neuropil is not as readily apparent as that of the laminar neuropil. The most obvious subdivision is into two strata: a posterior one which contains the branches of the M neurons (see Fig. 5a,b), and an anterior one that does not. The LMc neurons also terminate at these different levels in the medullary neuropil. For example, the type 2 LMc cells terminate in the posterior stratum and the type 1 LMc cells terminate in the anterior stratum. We do not currently have enough information to suggest any particular functional differences to correlate with the anatomical differences between these two strata of the medullary neuropil.

Other cell classes also show non-uniform distributions of their branches within the neuropils, but not segregation into the strata discussed above. For example, the L neurons branch mostly in the dorsal half of the laminar neuropil, but in all laminar strata. The LM neurons have few branches in the ventral half of the posterior stratum of the medulla but branch throughout the anterior stratum. The functional significance of these distributions also remains to be explored.

**Other characteristics of the classes of neurons found in the optic ganglion**

With the exception of the M class of neurons, which has not previously been reported, examples of neurons from each class were already drawn by Retzius (1906) from methylene-blue-stained whole mounts of *Daphnia.* The computer reconstruction of all the neurons in one hemiganglion also gives us, however, an idea of how many cells of each type are to be found in the OG.

We found differences between classes in the number of neurons. There is no obvious significance to the numbers except in the case of the LMc neurons, where the number (55) is a multiple of the number of columns (11) and hence of ommatidia as well. This number arises via the sequential recruitment of five LMc precursors by each ommatidial bundle of photoreceptor axons (LoPresti et al., '73). Although we have not studied the development of the other interneurons in the OG, the fact that classes of neurons other than the LMc cells do not have multiples of 11 neurons suggests that these classes do not arise in a repeating sequence similar to that of the LMc neurons. (As pointed out in Results, the number of neurons in the S class (11) is not meaningful, since it depends upon where the filmstrip ended.) However, four classes each contain 16–18 neurons, which suggests some kind of relationship or coordination in their development.

The positions of the cell bodies of the neurons belonging to each class are different for each class (see Fig. 2). However, only the LMc cell bodies occupy a region uniquely (see Fig. 2a,b). The cell bodies of other classes, while generally restricted to particular regions of the ganglionic cortex, show varying degrees of overlap among classes. For the most part the cell bodies of a particular class cluster together (e.g., the bilateral neurons, Figs. 2i,j), but in some cases they are more dispersed (e.g., the LM neurons, Fig. 2g,h). Whether or not such clustering is generally the result of how each class of neurons arises in development, as is the case for the LMc neurons (see Flaster et al., '82), remains to be investigated. A consequence of the overlap of the cell bodies of different classes, along with the fact that there is little difference in the diameters of most of the neurons in the OG, means that the class of a neuron cannot in general be ascertained by examination of its cell body (with the exception of the LMc neurons). In practical terms, this means that at least the initial association of a particular function to a specific neuron or class will require joint use of intracellular recording and dye-filling. A dye-filled cell can be examined in whole mount and easily identified with regard to class and, by comparison with the reconstructed neurons (which can be displayed at any orientation on the computer screen), also assigned an individual identity.

We found a close correlation between cell body position and the location of a cell’s branches for only one class, the M neurons. This is largely because these neurons have small branching fields close to their cell bodies, as can be seen in Figure 5c and d. A correlation is also found among the bilateral neurons, whose cell bodies are located in the same level of the ganglion as the commissures (see Figs. 2i, 7, 8). Some other classes showed weaker but interesting correlations between cell body position and cell branch locations. For example, the subset of LM neurons shown in Figure 6c,d branch in the laminar neuropil at a location diametrically opposite to that of their cell bodies.
We also examined various properties of the branching patterns of the OG neurons, such as field size and location of branching fields of individuals and their classes. With respect to size of branching field, we called a cell a small-field neuron if its branches in a neuropil were contained within three adjacent columns, and called it a large-field neuron if they extended further. Using these criteria, the M class is made up almost entirely of small-field neurons (see Fig. 5), the LMc class consists of small-field neurons by definition (they define the individual columns), and the rest of the classes are mixed. Particularly interesting are neurons in the LM and LMS classes, which branch in both laminar and medullary neuropils, and can have small- or large-field characteristics in both, or small-field in one and large-field in the other (see, for example, Figs. 6e,f, 11c–f).

Although specific hypotheses about the function of such interneurons must await determination of synaptic connectivity and physiological recordings, it is intriguing to consider their possible roles in the integration of topographic and other types of information. For example, Figure 11e and f show an interneuron which in the posterior half of laminar neuropil innervates columns 1 and k at the lateral edge of the ganglion, whereas in the medullary neuropil it innervates medial columns a.e, and f as well as more lateral columns (see Fig. 1 for definition of k and anna:); a large process also exits the ganglion, but what it does in the SEG is not known. It is possible that this cell could be comparing signals from two areas of visual space and conveying this information to higher centers.

As a final point, we have repeatedly found instances of specific pairs of neurons having major processes that travel through the neuropils together, although the two cells do not appear to be synaptically coupled. For example, within each column LMc cells of types 2 and 3 are directly apposed throughout almost the entire column and serve as an easily recognizable marker in the serial sections. A similar relation among LM neurons can be seen in Figure 6d. Whether these associations are functionally of any consequence, or are an epiphenomenon resulting from developmental dynamics, remains to be explored.

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LITERATURE CITED


