Synapses onto Different Morphological Types of Retinal Ganglion Cells

Abstract. *The percentage of bipolar and amacrine synapses onto ganglion cell dendrites of the ground squirrel has been determined by electron microscopy of cells impregnated by the Golgi method. One group of ganglion cells has mainly amacrine input (approximately 97 percent); the other group has an approximately equal bipolar and amacrine input. Morphologically distinct types of ganglion cells usually have a consistent synaptic input, but exceptions may exist.*

Electron microscopy of neurons impregnated with silver salts by the Golgi method is a potentially powerful method for the quantitative determination of synaptic contacts onto different types of neurons. However, fine structural preservation of tissue processed by the Golgi method has not previously been of adequate quality to allow such studies routinely, although several reports have described the use of Golgi impregnation coupled with electron microscopy to identify the location of processes within neural tissue (*1*). We have found that a twofold modification of the Golgi-Colonnier method results in a much improved fine structural appearance of the retina of the ground squirrel (*Citellus mexicanus*). Basically, these modifications are (i) postfixing with osmic acid after initial glutaraldehyde fixation, and (ii) a reduced staining time of 1 day each in potassium dichromate and silver nitrate (*2*). In such tissue, synaptic contacts onto silver-impregnated processes are well preserved and can readily be recognized (Fig. 1).

We have used this method to ask two questions concerning the ganglion cells in ground squirrel: (i) Do morphologically different ganglion cells have different synaptic inputs, and (ii) if so, can these quantitative differences in synaptic input be related to the physiological properties of the ganglion cells? The retina of the ground squirrel has been used, because Michael (*3*) showed that it possesses two major functional classes of ganglion cells [similar classes of ganglion cells have been described in the retinas of many vertebrates (*4, 5*)]. One class of ganglion cells in the ground squirrel responds tonically to retinal illumination; its receptive field can usually be mapped into center and surround areas that are mutually antagonistic. These cells are termed contrast-sensitive units. Other cells of this class show color opponent characteristics, and the antagonistic regions of these fields may overlap either partially or completely. The second class of ganglion cell in the ground squirrel responds phasically to spots of light presented to the center of its receptive field, and they have the additional property of responding vigorously to movement of a spot through the field center in a "preferred" direction. The receptive fields of these two classes of ganglion cells in the ground squirrel differ in a further respect; tonic cells tend to have larger center field diameters than phasic cells. This size difference provides a means of correlating the anatomy with the physiology of such ganglion cells.

Anatomical and physiological studies suggest that these two functional classes of ganglion cells may have different synaptic inputs. For example, intracellular recordings in the retina of the mudpuppy (*5*) have shown that amacrine cells show receptive field properties and phasic activity very similar to that of phasic ganglion cells. On the other hand, bipolar cells show receptive field properties and tonic activity similar to those of tonic ganglion cells. Because amacrine and bipolar cells provide the only input to ganglion cells, it has been proposed that phasic ganglion cells have mainly amacrine input, whereas tonic cells have a substantial bipolar input (*5*). The anatomy can now readily be correlated with the physiology by employing the improved fixation of Golgi-processed tissue since amacrine and bipolar synapses can be distinguished anatomically. Bipolar synapses are recognized by a presynaptic ribbon (Fig. 1a), amacrine synapses by a cluster of vesicles along the presynaptic membrane (*6*) (Fig. 1b).

Golgi-impregnated ganglion cells in the retina of the ground squirrel were studied by light microscopy in thick (50 μm), vertical sections and classified by the following criteria: (i) extent of dendritic spread, (ii) the level (or levels) in the inner plexiform layer in which the dendrites are confined, (iii) the diameter and texture of the dendrites, and (iv) the gross branching pattern of the dendrites. Cells were classified as different morphological types if they varied in one or more of these criteria. For example, cells were considered as separate types if their processes ran on different levels in the inner plexiform layer, even though their branching patterns were similar. Conversely, cells whose processes were contained within the same layer were classified separately if their branching patterns appeared different. With these criteria, we could identify at least 15 morphologically different ganglion cell types in the ground squirrel (*7*). Each of the classes appears distinct,

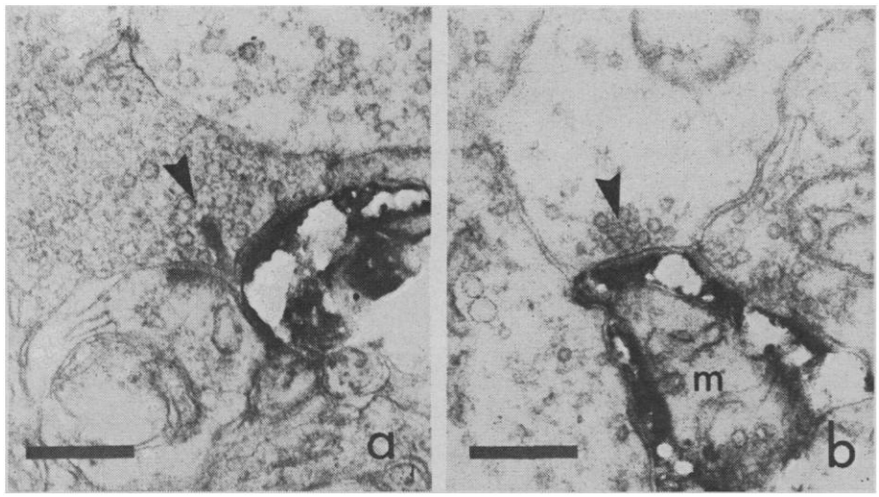

Fig. 1. Electron micrographs of synapses onto silver-impregnated ganglion cell dendrites in the retina of the ground squirrel. (a) Bipolar synapse. The ribbon in the bipolar terminal (arrow) is directed between two postsynaptic processes, one of which is a silver-impregnated ganglion cell dendrite. (b) Amacrine synapse. A cluster of synaptic vesicles closely opposed to the presynaptic membrane (arrow) marks an amacrine synapse onto an impregnated ganglion cell dendrite. With Golgi impregnation, silver salts are deposited throughout the cytoplasm but organelles such as mitochondria (*m*) are spared. Each marker equals 0.25 μm.

because little, if any, gradation between the types is observed. Examples of some of these types are shown in Fig. 2.

Well-isolated examples from each of these 15 types were subsequently thin-sectioned for electron microscopy to determine what percentage of their input was amacrine and bipolar. The smaller cells could usually be completely serially sectioned and as thoroughly inspected as desired. An equally thorough analysis of the larger cells was prohibitively difficult. So these were studied by serial sectioning through only the middle plane of their dendritic fields. Because some of these tended to have only sparse, long-ranging processes, the numbers of synapses counted in these cases were considerably less. No axosomatic contacts were observed; and on the stratified cells, very few synapses were found on dendrites between the perikaryon and their main levels of branching.

For simplicity we report here only the nine most commonly observed ganglion cells in our preparations (Table 1). Study of the less commonly stained cell types indicates that they also conform with the general pattern of results presented. Certain types of cells that have common morphological features and similar synaptic inputs are grouped together in Table 1. For example, types 2 and 3 are bistratified cells [that is, they have processes that run at two separate levels of the inner plexiform layer (see Fig. 2e)] and have similar dendritic spreads (60 to 70 μm), branching patterns, and very similar inputs. Nonetheless they were classified as distinct types of ganglion cells because their processes are confined to different zones of the inner plexiform layer.

Ganglion cells in ground squirrel can be grouped into two general classes with regard to synaptic input (Table 1). Group A has a very high percentage of amacrine input (~97 percent). Group B has an approximately equal frequency of bipolar and amacrine input, although one type in group B (type 1b) has a clear predominance of bipolar input. All of the amacrine-dominated group A have rather small dendritic fields (40 to 100 μm); on the other hand, two of the types of cells in group B have dendritic spreads of over 200 to 500 μm. Michael (*3*) determined the physiological sizes of receptive field centers for ganglion cells of the ground squirrel. Phasic cells have small receptive field centers (60 to 115 μm) matching well the dendritic spreads of the ganglion cells with primarily amacrine input. The tonic cells have receptive field centers ranging from 60 to 500 μm, matching reasonably well the dimensions of dendritic spreads of group B. We have termed the type with a 20-μm dendritic spread "midget" ganglion cells (Fig. 2c), because with their limited dendritic spreads they are likely candidates for receiving input from the midget bipolars we have observed in the retina of the ground squirrel that are postsynaptic to only a single cone. Since the midget ganglion cells in the ground squirrel are small, it is possible that their receptive fields

Table 1. Synaptic input onto different types of ganglion cells of the ground squirrel. For purposes of ganglion cell classification, we have arbitrarily divided the inner plexiform layer into six equally spaced layers ~ 10 μm thick), numbered from the distal to proximal borders. The number of the layer or layers to which the dendrites of a cell are confined is given in the column headed "Layering." Abbreviations: Mono, monostratified; Bi, bistratified.

Type	Cells studied (No.)	Average input		Range of bipolar input (%)	Synapses observed (No.)	Dendritic morphology		
		Amacrine (%)	Bipolar (%)			Description	Layering	Maximum lateral spread (μm)
				Group A				
1a	3	100	0	0–0	203	Mono	3	40–50
2 and 3	3	96	4	3–5	212	Bi	1 and 3, 2 and 5	60–70
6–8	3	95	5	4–7	132	Mono	1, 2, 4	90–100
Average		97	3					
				Group B				
1b	2	22	78	73–79	46	Mono	3	40–50
9	3	54	46	33–57	123	Midget	4	20
11	2	53	47	44–49	40	Mono	4	> 200
13	3	52	48	47–50	27	Mono	4–5	> 500
Average		46	54					

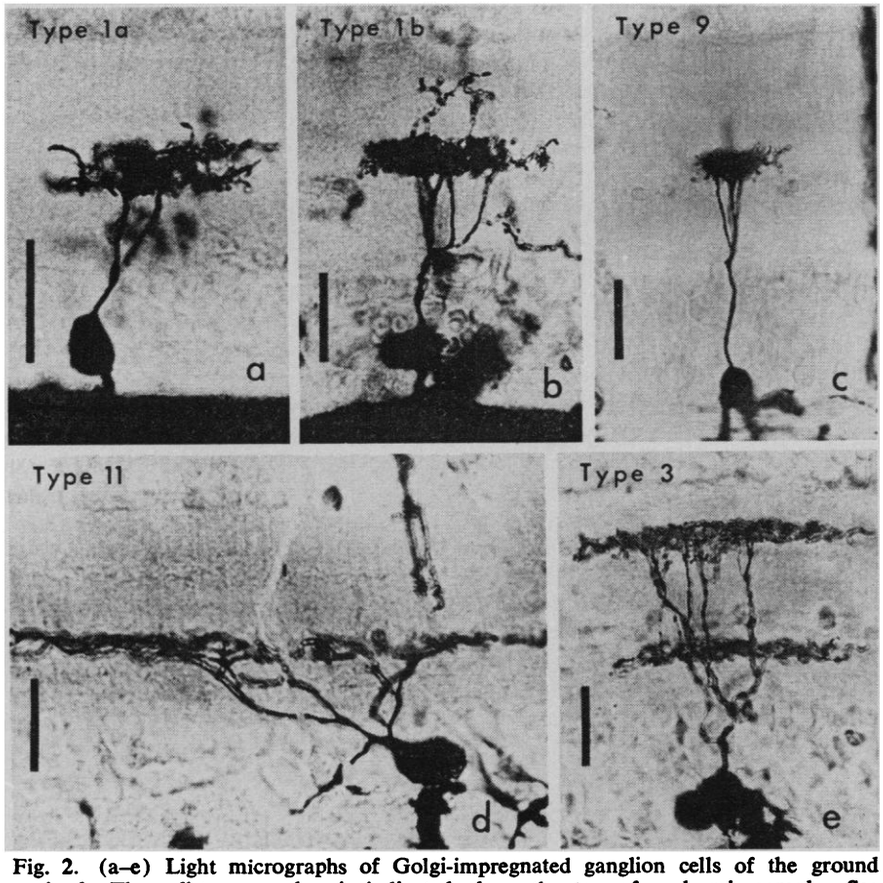

Fig. 2. (a–e) Light micrographs of Golgi-impregnated ganglion cells of the ground squirrel. The cell-type number is indicated along the top of each micrograph. See text and Table 1 for discussion and description of the cells. Each marker equals 30 μm.

have not as yet been detected, explaining their absence in Michael's data.

Each of the types of ganglion cell so far studied in replication has been found to have a rather consistent ratio of amacrine to bipolar input (Table 1). For example, three cells of type 1a have been carefully examined. All have shown only amacrine input. No suggestion of any bipolar input onto these cells has been observed even though one of these was examined in its entirety, section by section. Onto this cell, 95 amacrine synapses were observed, which may represent at least half of the synapses onto that cell (*8*). Because the synaptic ribbon of the bipolar synapse is easy to detect, we are confident that this cell type has no bipolar input. Such ganglion cells are of particular interest, because they indicate that some ganglion cells have exclusively amacrine input and are, therefore, at least fourth-order neurons along the visual pathway in the sequence of receptor to bipolar to amacrine to ganglion cell. Although it has been previously proposed that ganglion cells may receive only amacrine input (*9*), this is the first direct demonstration of this. On the other hand, the other cell types in group A (types 2, 3, and 6 to 8) always have some bipolar input even though the overwhelming majority of their input is from the amacrine cells. Provided enough sections are examined, a small bipolar input of about 5 percent can always be detected on the cells belonging to these types.

It thus appears that a particular morphological type of cell, as presently defined, has a ratio of amacrine and bipolar synaptic input that is consistent from cell to cell. However, we have found so far one possible exception to this generalization. Cell types 1a and 1b appear to us morphologically indistinguishable in the light microscope (Fig. 2, a and b). Their dendrites are found at the same level in the inner plexiform layer, they appear to have a similar branching pattern and dendritic spread; yet they have different synaptic inputs (100 percent amacrine, 0 percent bipolar input compared to 22 percent amacrine, 78 percent bipolar input). So far we have been able to distinguish between these cells only on the basis of their synaptic inputs (*10*). The five cells examined could be assigned unequivocally to one class, or another, after even preliminary electron microscopic study. No examples with an intermediate ratio of synaptic input have been seen, and it is interesting that these two light microscopically identical cells are so dissimilar with regard to their synaptic input.

Two conclusions follow from our results. (i) Ganglion cells in the retina of the ground squirrel can be divided into two main classes based on synaptic input. One class receives the overwhelming majority of its input from amacrine cells. The dimensions of the dendritic spreads of these cells suggest that they are the phasic ganglion cells. The second class of ganglion cell receives a substantial bipolar input ranging from 46 to 78 percent of its total input. The dimensions of these cells best match those of the tonic ganglion cells. This is in accord with the suggestion that phasic cells receive the bulk of their input from the amacrine cell system, whereas contrast-sensitive and opponent-color cells receive a more substantial bipolar input. In the ground squirrel some cells may have no bipolar input, but there are no ganglion cells without amacrine input. (ii) Morphologically distinct types of ganglion cells have consistent synaptic inputs so that input ratios can now be predicted from light microscopic observations. However, there may be exceptions to this rule. Certain ganglion cells in the ground squirrel have appeared so far morphologically identical by light microscopy, yet electron microscopy reveals they have very different inputs. At the moment, we must further conclude that it is not always possible to correlate dendritic morphology of ganglion cells with a particular synaptic input. Thus, form, or at least level of dendritic branching of a ganglion cell, may not determine its connectivity as is often presumed (*11*). That level of dendritic branching in the inner plexiform layer does not strictly determine the synaptic input to a cell is indicated also by the finding that other types of ganglion cells have processes that run in the same strata of the inner plexiform layer but have rather different synaptic inputs (for example, types 8 and 11; see Table 1).

It is difficult to understand why there are so many variations in ganglion cell morphology in the ground squirrel. Only a relatively few physiological types of ganglion cells have been described in the retina of the ground squirrel so far, and many of the morphologically distinct ganglion cells have a very similar ratio of amacrine to bipolar synaptic input. We wonder if the rich variety of dendritic layering, spread, and branching pattern of ganglion cells that has received so much study over the years reflects an equivalent variety of physiological types. From the results of our study, this appears not to be the case.

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References and Notes

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2. The tissue is fixed for 1 hour in cold 2.5 percent glutaraldehyde in sodium cacodylate buffer (0.065*M*, with 0.05 percent $CaCl_2$ added) and postfixed in 2 percent osmic acid in barbital–acetate buffer for 1 hour in the cold and for 30 minutes more while warming to room temperature (100 ml of osmic acid fixative can be made by adding to 36 ml of H_2O, 0.577 g of sodium barbital, 0.23 g of sodium acetate, 12 ml of 0.1*N* HCl, 2.25 g of sucrose, 2 ml of 1 percent $CaCl_2$, and 50 ml of 4 percent OsO_4). The tissue is then transferred for 1 day to 3 percent potassium dichromate (with no other fixative) and for 1 day to 0.75 percent silver nitrate. Serial thin sectioning was made possible by a technique [R. W. West, *Stain Technol.* **47**, 201 (1972)] that allows initial thick sectioning of very hard Epon.
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6. J. E. Dowling and B. B. Boycott, *Proc. Roy. Soc. London Ser. B* **160**, 80 (1966).
7. Although it is easily seen that the superior retina of the ground squirrel is much thinner than the inferior retina [P. G. Vaidya, *J. Comp. Neurol.* **122**, 347 (1964)], we have been able to find most of the ganglion cell morphologies in both superior and inferior retina. Also, most of the cell types are seen in both central and peripheral retina.
8. As many as half of the synapses may have been missed because synapses poorly oriented within the section could not be recognized. Also, the slightly inferior fixation resulting from the Golgi procedure makes synapses more difficult to observe.
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10. Details of branching pattern are more easily studied in flat section, however, and it is possible that small but significant differences in branching pattern may be overlooked in vertical sections. We are undertaking a more detailed study of dendritic tree morphology of these ganglion cells employing flat sections.
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