

THE GIANT SEROTONERGIC NEURON OF *APLYSIA*: A MULTI-TARGETED NERVE CELL¹

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Abstract

We have examined the various classes of cells that can be innervated by the giant cerebral neuron (GCN), an identified serotonergic cell that functions in arousal and maintenance of feeding behavior. We have found that this single neuron innervates a remarkable variety of postsynaptic targets by means of varicosities bearing active zones. The neuron's presynaptic terminals were identified by electron microscopic radioautography after intrasomatic injection of a tritiated amino sugar precursor of membrane glycoproteins; these are moved to nerve endings by fast axonal transport. In addition to endings on buccal muscle, we have found that GCN forms appositions with the morphological characteristics of synapses on axonal processes and cell bodies of neurons in the buccal ganglion and, unexpectedly, it forms appositions most often with glial cells which form the lining of intraganglionic hemal sinuses. Thus, GCN, through contacts on a variety of postsynaptic targets, has the potential of mediating several different functions, each of which is usually associated with a specific specialized type of neuron. In random electron micrographs, approximately 14% of GCN's varicosities had membrane specializations presumed to be the sites where transmitter is released. In these sections, GCN's active zones were quite small, 0.25 μm or approximately five vesicle diameters long. One of GCN's terminals was reconstructed completely from a series of thin sections. It had a single, flat ovoid active zone with an area of 17 μm^2 . We suggest that active zones often are overlooked in random sections of monoaminergic terminals because they are small.

As a class, neurons innervate a great variety of target cells (other neurons, muscle, glands, blood vessels, or glia); but is an individual neuron restricted in its range of postsynaptic targets? For example, can one branch of a neuron contact blood vessels to serve a neurosecretory function and other branches contact other neurons to mediate rapid synaptic communication? To answer this question, one needs to label a single cell and to examine its contacts systematically. We have approached the question of postsynaptic target heterogeneity using the giant cerebral neuron (GCN), an identified serotonergic cell with known behavioral functions—arousal and maintenance of feeding behavior in *Aplysia* (Weiss and Kupfermann, 1976; Weiss et al., 1978) and other opisthobranch molluscs (Gillette and Davis, 1977; Gelperin, 1981; Granzow and Rowell, 1981).

The general morphology of serotonergic terminals has been studied in the central nervous system of vertebrates and in other invertebrates (for references, see Shkolnik and Schwartz, 1980). We have described the variety of vesicle types in the giant cerebral neuron previously and have suggested a coherent maturational sequence that relates the synaptic vesicles observed in the terminals to those in the proximal axon and cell body of this identified *Aplysia* neuron (Shkolnik and Schwartz, 1980).

GCN has been shown electrophysiologically to provide synaptic action to follower neurons in the buccal ganglion and modulatory action through its peripheral branches to muscle. We now have extended the survey of GCN's synaptic terminals by examining the buccal ganglion with electron microscopic radioautography after injecting *N*-[³H]acetylgalactosamine into the neuron's cell body in the cerebral ganglion. Membrane glycoproteins, labeled by incorporation of the ³H-sugar precursor, move rapidly by fast axonal transport and serve to mark terminals. Numerous labeled terminals were found in all regions of the ganglion. This widespread distribution suggests that GCN can innervate a remarkable variety of postsynaptic targets. Although the varicosities come into extensive contact with these components throughout the ganglion, evidence of synaptic junctions is rare. Nevertheless, we

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have shown that some of the identified varicosities possess synaptic membrane specializations, indicating the presence of active zones.

Materials and Methods

The central nervous system was excised from three specimens of *Aplysia californica* weighing 40 to 70 gm and maintained in a chamber filled with an artificial seawater containing glucose, amino acids, and vitamins (Eisenstadt et al., 1973). A giant cerebral neuron was injected with *N*-[³H]acetylgalactosamine (25 Ci/mmol, New England Nuclear Corp., Boston, MA) as previously described (Ambron et al., 1980). After the injection, the nervous tissue was kept for 15 to 20 hr at 15°C to permit sufficient radioactive membranes to be transported from the cell body along the cerebrobuccal connective to label terminals in the buccal ganglion at a distance of 1 to 1.5 cm.

Preparation of tissue for microscopy. Tissue was fixed in 6% (v/v) glutaraldehyde in 0.2 M *sym*-collidine (pH 7.4) made 0.7 M in sucrose and 0.7 mM in CaCl₂, postfixed in 1% (w/v) OsO₄ in 0.8 M sucrose and 50 mM sodium phosphate (pH 7.3), and embedded in Epon as previously described (Thompson et al., 1976; Goldman et al., 1976; Ambron et al., 1980; Shkolnik and Schwartz, 1980). For light microscopic radioautography, 2- μ m sections were mounted on glass slides, which then were coated by dipping in L4 Ilford emulsion (Polysciences, Inc., Warrington, PA), diluted 1:1, and exposed for 1 to 3 weeks at room temperature. Radioautographs were developed for 4 min at 20°C in Kodak D-19 developer and fixed in Kodak acid fixer. Sections were examined by bright-field and phase contrast microscopy.

For electron microscopic radioautography, sections, 80 to 100 nm (determined by interference color), were mounted on Formvar-coated single slot grids and coated with a monolayer of the emulsion (diluted 1:4) applied with a platinum wire loop. The coated grids were exposed for 4 to 12 weeks, developed for 3 min in Kodak Microdol-X, and stained for 6 to 8 min with saturated uranyl acetate and for 1 to 3 min with lead citrate. Sections were examined in a Philips EM-301 electron microscope.

Identification of GCN's terminals. Grids containing sections of the buccal ganglion were scanned systematically under low power to locate silver grains. A profile was attributed to the injected GCN if it contained at least three silver grains or if the same profile was labeled in at least two adjacent sections. Background labeling was essentially negligible as indicated by the absence of grains over neuronal nuclei or over regions of the grids not containing tissue but coated with emulsion. In addition, previous analysis of the distribution of silver grains over the cell body and proximal axon of GCN after injection of *N*-[³H]acetylgalactosamine showed that the label is confined to the injected neuron (Ambron et al., 1980).

About 600 micrographs containing labeled profiles were studied; these represented approximately 200 individual varicosities from all regions of the buccal ganglion. One varicosity was reconstructed completely from a series of consecutive thin sections using the methods of Macagno et al. (1979) at Columbia University's Graphics Computer Facility.

Results

First, we will discuss the general morphology of GCN's terminals; next, present evidence for the presence of active zones; and last, characterize GCN's postsynaptic targets.

General morphology of GCN's terminals. Electron microscopy has revealed that GCN's terminals consist of varicosities connected by thin intervaricose segments (Shkolnik and Schwartz, 1980). Numerous branches were found in all three regions of the ganglion: neuropil, cell body layer, and glial capsule (Fig. 1). Chains of varicosities are numerous throughout the neuropil (Fig. 2). They course between large nerve cell bodies, frequently appearing to penetrate *into* somatic cytoplasm (Fig. 3A). The chains also reach out to the external margin of the ganglion where the glial capsule lies beneath the connective tissue sheath (Fig. 3, B and C).

GCN's varicosities are ovoid structures with a long axis of 2 to 3 μ m and a short axis of 1.5 to 2 μ m. Frequently they appear to be distorted by surrounding structures. Thus, at the margin between the cerebrobuccal connective and the ganglion and in the neuropil, the varicosities were seen mostly as large polygonal profiles whose shapes appear faceted to conform to adjacent axons of other neurons (Fig. 2B). Another variation in the shape of the varicosity also was observed in invaginating axosomatic synapses that occur in the cell body layer to be described below. Similar axosomatic contacts made by L29, a putative serotonergic neuron of the abdominal ganglion of *Aplysia* now also have been described by Bailey et al. (1981a). Still another frequent variation occurs when two of the neuron's varicosities run together, one nestling in the other (Fig. 2, C and D).

We found no indication for synaptic input onto the labeled terminals or intervaricose neurites of GCN. Unlike the varicosities of L29 described by Bailey et al. (1981a), none of the varicosities of GCN had spine-like projections for receiving input from other neurons.

Active zones. In one instance, we were fortunate to have an almost complete series of 27 consecutive sections through a labeled terminal in the cell body layer of the buccal ganglion that enabled us to reconstruct the varicosity. Three views of the computer-assisted reconstruction are presented in Figure 4. We measured the perimeter of the labeled profile in each section (Fig. 5). The widest part of the varicosity, with a long axis of 2.5 μ m and a short axis of 1 μ m, is quite close to one end. The length of the varicosity (the distance between the two intervaricose segments) can be estimated to be 2.5 to 2.7 μ m from the number of sections containing profiles of the varicosity. The total surface area of the varicosity was 10.8 μ m².

Although labeled varicosities come into extensive contact with axons, neuronal cell bodies, and glia throughout the ganglion, evidence of synaptic contact is rare. The complete set of morphological specializations that define active zones have not been visualized easily in the nervous system of *Aplysia* (see Coggeshall, 1967), but recently Bailey et al. (1979, 1981b), Graubard (1978), and Tremblay et al. (1979) have shown quite clearly that they exist. We have used their criteria to recognize an active zone; these include: the presence of (1) a focal presynaptic

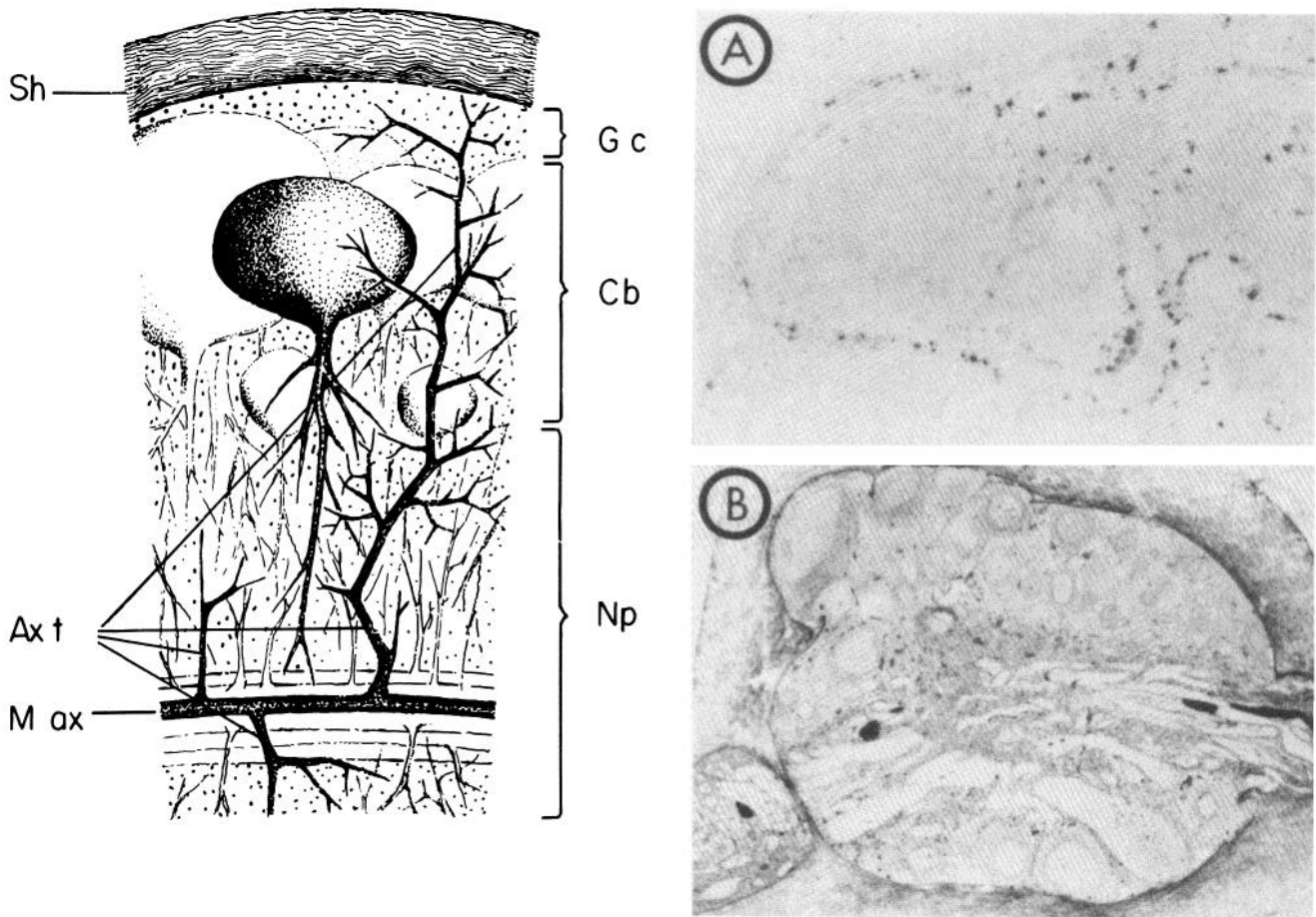


Figure 1. The distribution of GCN's terminals in the three regions of the buccal ganglion. The distribution of GCN's axon terminals (*Ax t*) is shown in the diagram of a horizontal segment of the buccal ganglion. The main axon (*M ax*) enters the ganglion in the cerebrobuccal connective and passes through the ipsilateral hemiganglion to exit through the commissure leading to the contralateral hemiganglion. As it passes through the hemiganglion, it gives off numerous branches which ramify within the neuropil (*Np*) and cell body layer (*Cb*). Terminal branches reach the glial capsule (*G c*) that lies underneath the connective tissue sheath (*Sh*) covering the ganglion. **A**, Light microscopic radioautogram showing the arrangement of GCN's varicosities that form a basket-like network around cell bodies of unidentified neurons. The tissue is unstained. Magnification: $\times 590$. **B**, Light microscopic radioautogram of a horizontal section of a buccal hemiganglion with cerebral-buccal connective and commissure. GCN's main axon is seen labeled in an oblique section of the connective on the *left* and within the ganglion after it enters the neuropil and crosses to exit in the commissure from the *right*. Magnification: $\times 160$.

accumulation of small, electron-lucent vesicles; (2) pre-synaptic densities that often give the impression of being pyramidal in shape; (3) a length of apposed external membranes that are straightened; and (4) a widened extracellular space between the pre- and postsynaptic membranes containing electron-dense amorphous material. As previously noted (see Bailey et al., 1979; Tremblay et al., 1979; Colonnier et al., 1979), the well defined special cytoplasmic density bordering the postsynaptic membrane that is seen often in vertebrate synapses is not prominent in putative synapses of *Aplysia*.

In 27 labeled varicosities, we identified an active zone in only a few adjacent sections (Table I). These had an average length of 251 nm. In some single sections, all four of the characteristic specializations were clearly present. In others, one or another of the criteria were absent in individual sections. In these instances, we counted a region of membrane as being an active zone only if all of

the criteria appeared in adjacent sections. For example, clefts are not widened in the sections shown in Figures 6A and 7A but do appear to be widened in the adjacent sections shown in Figures 6B and 7B. Likewise, in some sections, vesicle accumulations are prominent, but in neighboring sections, vesicles are scarce or absent (see Fig. 5). Thus, not all of the four criteria were met in every section; each series, taken together, however, enabled us to distinguish an active zone from images (like membranes cut tangentially) that might be suspected of being a synapse.

In one additional varicosity, we were able to reconstruct an active zone from consecutive sections (Figs. 4 and 7). The lengths of the specialization and the numbers of vesicles clustering within 50 nm of the straightened membrane are plotted in Figure 5. The shape of this putative release site, the only one found in the reconstructed varicosity, is an oval disc measuring 409 nm

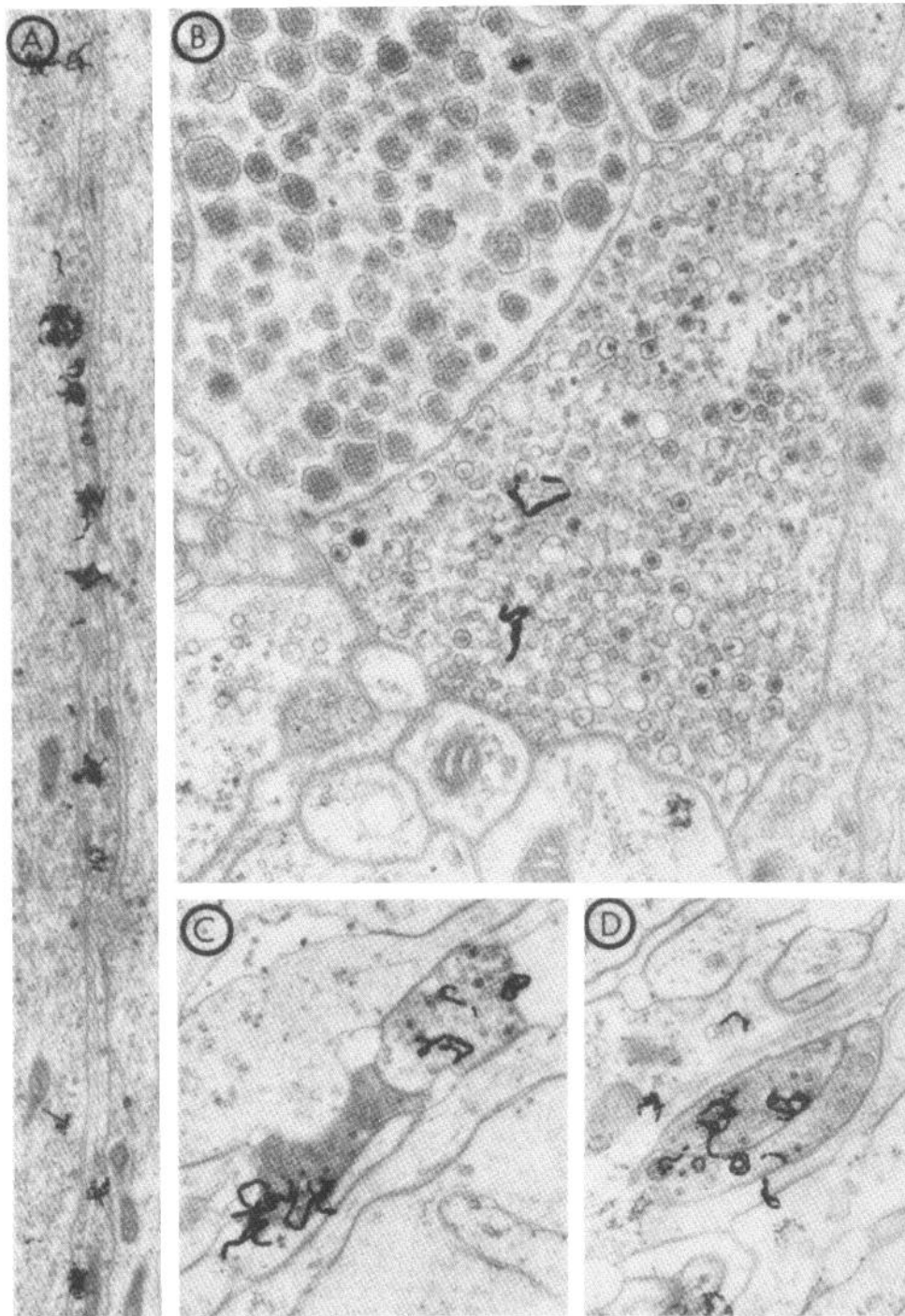


Figure 2. Varicosities of GCN in the neuropil region of the buccal ganglion. **A**, A chain of four labeled varicosities connected by intervaricose segments running between large axons of unidentified neurons. Magnification: $\times 14,000$. **B**, A large ($2\text{-}\mu\text{m}$ in diameter) varicosity. Note prevalence of large electron-lucent and dense core vesicles. Magnification: $\times 40,000$. **C** and **D**, Pairs of labeled varicosities often run together with each other. Magnifications: $\times 21,000$.

across at its widest point and extending through six sections, each approximately 80 to 100 nm thick. We estimated the surface area of this zone of apposition to be $0.17\ \mu\text{m}^2$, approximately 1.5% of the total surface area of the varicosity. The dimensions of this apposition are consistent with the short lengths of the 27 active zones

seen in random sections (Table I). GCN's active zones may appear so infrequently because of their small size.

Characterization of GCN's postsynaptic targets. The 28 identified active zones within the buccal ganglion appeared to contact three types of postsynaptic targets (Table I). Only a small proportion of these junctions

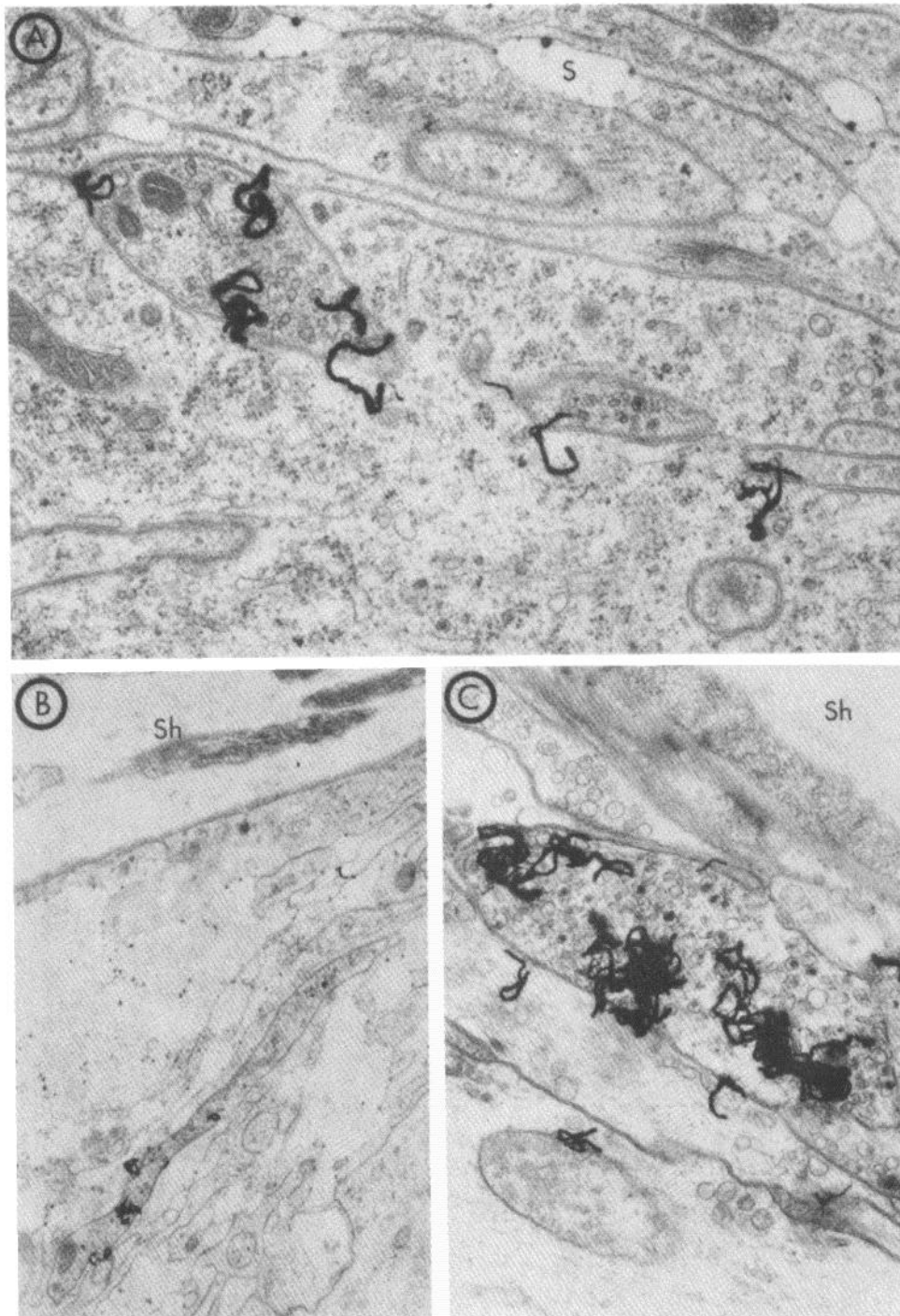


Figure 3. Varicosities of GCN in the cell body layer and in the glial capsule of the buccal ganglion. **A**, A series of three labeled profiles representing a snake-like process invaginating into the cytoplasm of an unidentified neuron. Note proximity of intercellular sinuses (*S*). Magnification: $\times 31,000$. **B**, A longitudinal section showing a labeled process extending almost to the margin of the glial capsule, which is covered by the connective tissue sheath of the ganglion (*Sh*). Magnification: $\times 10,000$. **C**, A labeled varicosity in the glial capsule just beneath the connective tissue sheath. Magnification: $\times 22,000$.

were onto axons, the typical synaptic contact found in invertebrate nervous tissue. The few axoaxonic contacts were found exclusively in the neuropil (Fig. 6, *A* and *B*).

Next most prevalent were synapses on cell bodies of unidentified, large neurons (Fig. 6, *C* and *D*); these often

invaginated deeply into the cytoplasm (Fig. 6*C*). Common in vertebrates, synapses on neuronal cell bodies are rare in invertebrates. Schacher et al. (1979) have described an axosomatic contact on a developing neuron in the embryonic abdominal ganglion of *Aplysia* which

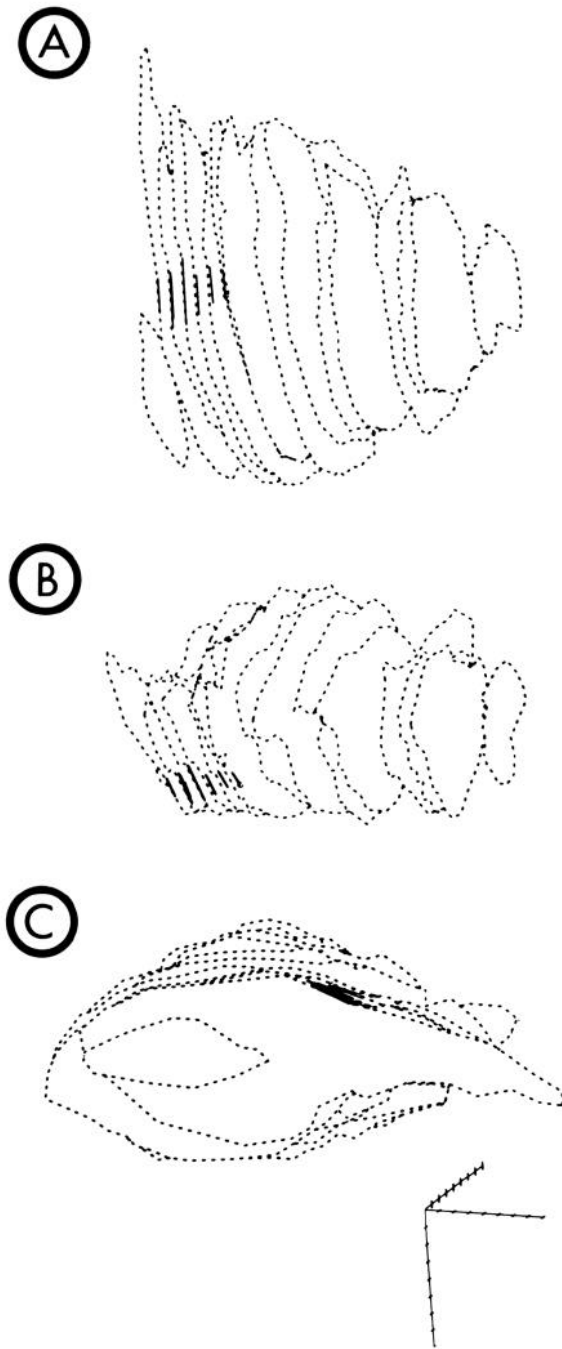


Figure 4. Three-dimensional reconstruction of a labeled varicosity of GCN in the cell body layer of the buccal ganglion from a series of consecutive electron micrographic sections. Three views of the computer-assisted reconstruction (see text) are shown: *A*, broadside; *B*, thin side; and *C*, end on. The locations of membrane specializations (active zone) are indicated by heavy solid lines. An interval on the three-dimensional coordinates (*inset*) represents $0.1 \mu\text{m}$.

disappears when the animal reaches maturity. To our knowledge, we are the first to observe them in mature *Aplysia* (Shkolnik and Schwartz, 1980), although axosomatic synapses have been found in cerebral ganglia of other gastropod molluscs (Nicaise et al., 1968; Zs.-Nagy and Sakharov, 1969, 1970). Axosomatic synapses may be

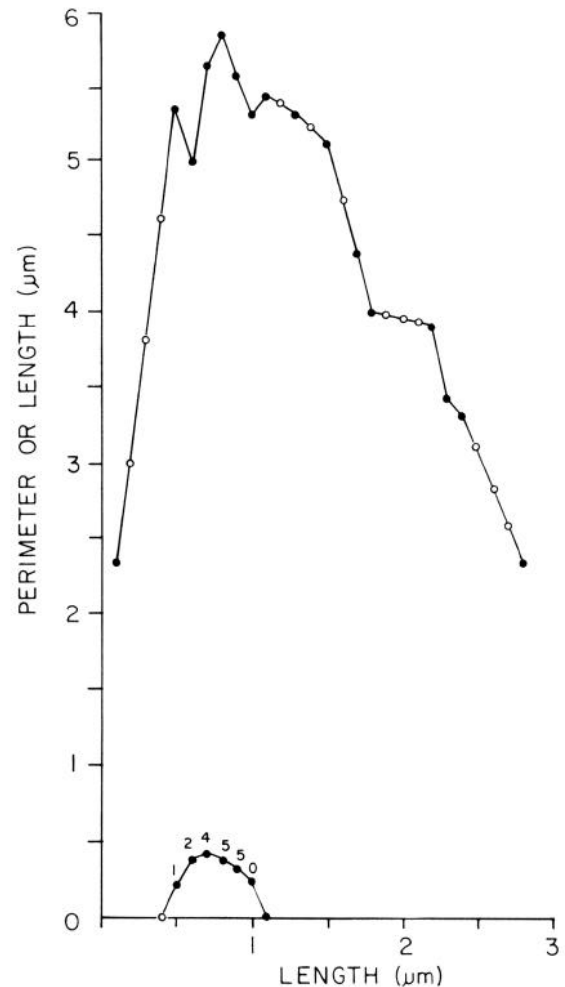


Figure 5. Dimensions of an identified terminal of GCN in the buccal ganglion. With a map measurer, we measured the length of the active zone (*lower curve*) and the perimeter (*upper curve*) of the labeled profile in electron micrographs (magnified $\times 51,000$) in the series of consecutive sections used for the reconstruction presented in Figure 4. These values are plotted along the abscissa with the assumption that the sections are $0.1 \mu\text{m}$ thick and uniform. The number above each point on the *lower curve* showing the length of the active zone represents the number of vesicles in each section at a distance of no more than one vesicle diameter (50 nm) from the synaptic membrane. There are several gaps in the series, due to lost sections, which are indicated by open circles on the *upper curve*. Most important is the gap between the end of the varicosity on the left and the active zone. Although the parameters obtained from the next six serial sections (which contain the active zone) suggest that the zone does not continue into the lacuna, the boundary on the left cannot be drawn definitively because the sections were not recovered.

quite general for serotonergic cells in molluscs: Bailey et al. (1981a) have noted subsequently that L29 has similar endings onto cell bodies of neurons in the abdominal ganglion of *Aplysia*.

Surprisingly, the large majority of GCN's appositions with the morphological characteristics of synapses were onto glial cells and their processes (Table I and Figs. 7 and 8). These contacts on glial cells occurred in all three

TABLE I

Distribution of elements postsynaptic to the giant cerebral neuron in the buccal ganglion of *Aplysia*

Postsynaptic Component	Frequency	Number of Active Zones	Length of Specialization	Number of Vesicles
	%		nm	
Glial cells	64	18 ^a	257 ± 19	3.5 ± 0.5
Neuronal cell bodies	25	7	230 ± 26	2.6 ± 0.5
Axons	11	3	266 ± 17	2.7 ± 1.5
Total		28	251 ± 14	3.2 ± 0.4

^a One of the 18 is the active zone from reconstructed varicosity (Fig. 4). Values of length and number of vesicles within 50 nm of the membrane from this active zone were not included in calculating the means ± SE in this table.

regions of the ganglion. Criteria for identifying the postsynaptic cell as glial were the presence of cytoplasmic organelles (endoplasmic reticulum, Golgi apparatus, and ribosomes) together with a characteristic nucleus containing dense heterochromatin distributed just beneath the nuclear membrane. These glial cells were of two types, containing finely dispersed granular material either with bundles of tonofibrils (dark glial cells; Fig. 7, A and B) or with clumps or aggregates of densely staining particles previously identified as glycogen (see Tremblay et al., 1979; Colonnier et al., 1979) (clear glial cells; Fig. 8A). Recently, Colonnier et al. (1979) have described synaptic contacts from unidentified axons on both types of glial cells in the abdominal ganglion of *Aplysia*, and Nicaise (1967) described junctions on interstitial cells in the peripheral nervous system of the Mediterranean sea slug, *Glossodoris*.

All of the synapses of GCN on glial cells neighbored sinuses that appear to be part of a continuous fluid space within the ganglion (Rosenbluth, 1963; R. S. Goldstein and J. H. Schwartz, unpublished experiments). Most prominent in the glial capsule, this system of narrow intercellular channels separates adjacent glial cells from one another. These spaces also can be seen extending between glial cells in the cell body layer (Figs. 3A, 6D, and 8, B and D) and in the neuropil (Figs. 2, C and D, and 8C). Synaptic junctions on glia in the cell body layer and neuropil also were associated directly with these channels. Particularly dramatic examples are shown in Figure 8 where the labeled terminals appear to be encircled by the intercellular channels or to abut directly on them.

Discussion

Synaptic specialization in central monoaminergic terminals. It has been argued often that central monoaminergic terminals lack active zones (see Groves, 1980). Synaptic specializations have previously been found to be absent or extremely infrequent in serotonergic endings. For example, in the rat locus ceruleus, Leger and Descarries (1978) reported that fewer than 10% of the varicosities, identified radioautographically after ventricular perfusion of [³H]serotonin, contained morphologically defined synaptic junctions; they concluded that this small proportion was far less than the proportion in the

same micrographs of unlabeled (non-serotonergic) terminals with active zones would have led them to expect. Recent studies of central noradrenergic (Olschowka et al., 1981; Takahashi et al., 1980) and dopaminergic (Groves, 1980) neurons indicate that central monoaminergic terminals are endowed with synaptic specializations.

The presence of sites specialized for the release of transmitter substance is of obvious functional significance since it is likely to contribute critically to determining how directed the action of the terminal can be. Thus, does GCN release serotonin onto specific postsynaptic targets or is the transmitter shed throughout the buccal ganglion at any point along a varicosity? Our morphological results are consistent with the idea that serotonergic terminals do contain synaptic specialization and suggest that active zones may appear so rarely in random sections because the zones constitute a relatively small proportion of the total surface area of the varicosity. We found that approximately 14% of the labeled varicosities of GCN appear with active zones in random micrographs, a somewhat greater proportion than Leger and Descarries (1978) found in the rat locus ceruleus.

The appositions displayed five of the six morphological features recognized as characteristic of synapses in other animals (see Dyson and Jones, 1980; Vrensen et al., 1980) and typical of *Aplysia* junctions that have not been stained specially (Bailey et al., 1979, 1981a; Tremblay et al., 1979). We identified active zones by the presence of (1) a presynaptic accumulation of small electron-lucent vesicles (these accumulations often stand out by contrast to the population of larger lucent vesicles and dense core vesicles that are characteristic of regions within the varicosities at a distance from the active zones), (2) presynaptic densities that may represent dense projections, (3) a stretch of closely apposed external membrane which is straightened, (4) a widening of the extracellular space between the pre- and postsynaptic cell, and (5) a synaptic gap containing electron-dense material.

Bailey et al. (1979) completely reconstructed four varicosities of an identified sensory neuron in the abdominal ganglion of *Aplysia* and found that two of the varicosities had two active zones each and two had only one. As shown in Table II, in which GCN's varicosity is compared to those of the sensory neuron, GCN's active zone is quite small, constituting a relatively small proportion of the varicosity's total surface area. Even though this comparison is made with only one serial reconstruction, the values obtained are supported by data from the 27 other active zones found in random sections (Table I). The range of dimensions of *Aplysia*'s sensory active zones is quite similar to those reported for central vertebrate synapses (see Dyson and Jones, 1980). If active zones of monoaminergic terminals tend to be small and occupy only a small proportion of the varicosity's total surface area, this would contribute to the rarity of finding membrane specializations in random sections.

Another feature of *Aplysia* synapses also could have contributed to the apparent rarity of active zones in our material. Bailey et al. (1981b) found that *Aplysia* synapses differ from those of vertebrates in having shorter dense projections and more irregular presynaptic grids. Nevertheless, examination of profiles in thick sections of

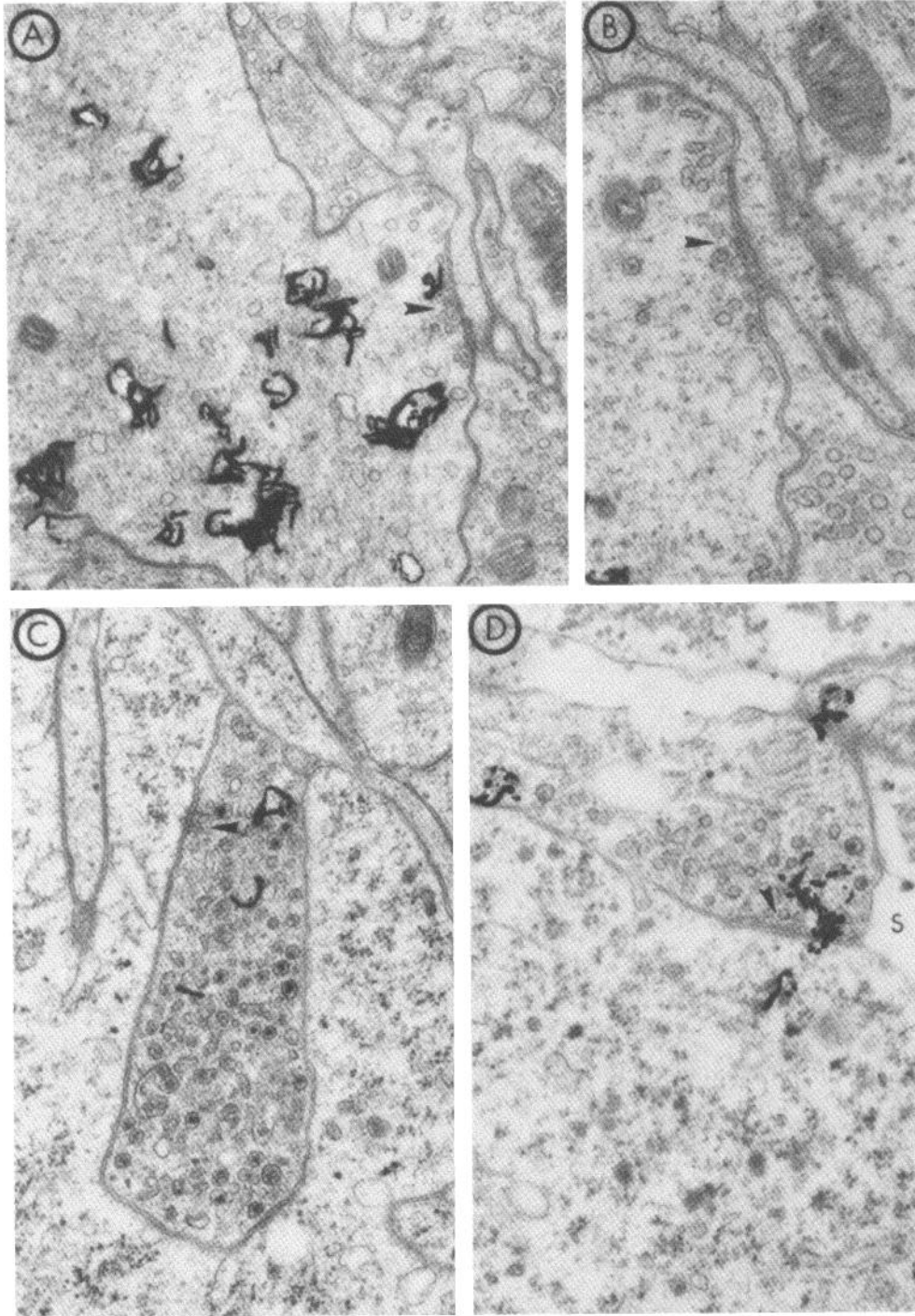


Figure 6. Axoaxonic and axosomatic synapses of GCN. *A*, A large (6- μm in diameter) axon in the neuropil region of the buccal ganglion that contains an active zone *en passant* (arrowhead). Note the scarcity of vesicles in the axon but the clustering of small, electron-lucent vesicles at the presumed site of membrane specialization. *B*, A section, serial to the one shown in *A*, in which membrane specialization, especially widening of the cleft, is evident (arrowhead). Magnifications: $\times 23,500$. *C*, A synaptic varicosity showing an active zone (arrowhead) invaginating into the cytoplasm of an unidentified nerve cell body. Despite the prevalence of large electron-lucent and dense core vesicles in the varicosity, the vesicles clustering at the zone of membrane specialization are small and electron lucent. Magnification: $\times 31,000$. *D*, Another labeled terminal of GCN synapsing (arrowhead) on the surface of a neuronal cell body. A sinus borders the active zone (S). Magnification: $\times 33,000$.

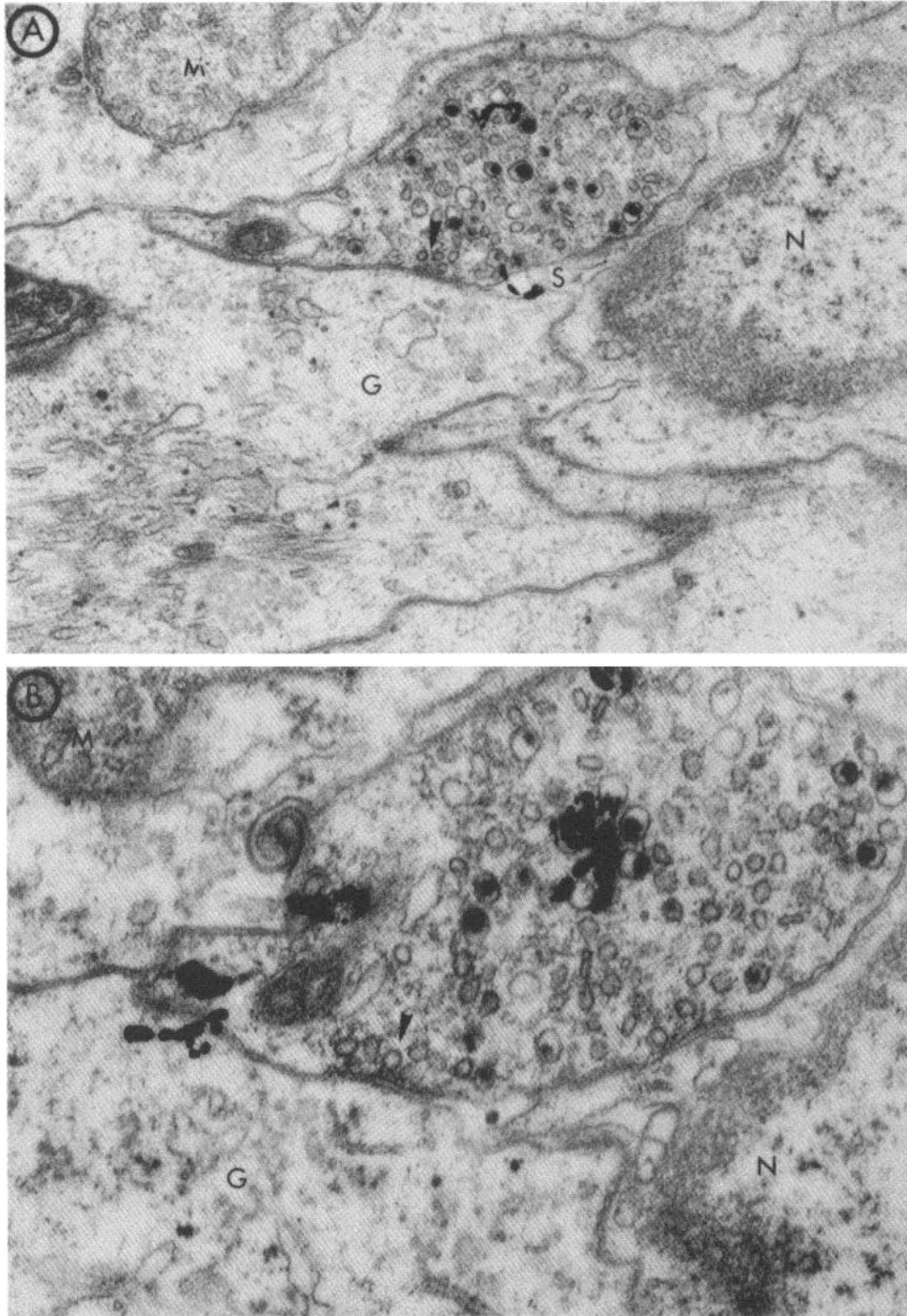


Figure 7. Axoglia synapses of GCN. A varicosity synapsing on the body of a dark glial cell (*G*). This micrograph is part of a set of consecutive sections that permitted reconstruction of the varicosity (see Figs. 4 and 5). Profiles of this varicosity were labeled in each section. The active zone (*arrowhead*), which was present in six consecutive sections, is close to an intercellular lacuna (*S*). The glial nucleus (*N*), with a characteristic accumulation of heterochromatin along its perimeter, is part of the cell containing abundant tonofibrils and diffuse, dark granular material; in adjacent sections, the cytoplasm of the postsynaptic region can be shown to be continuous with the cytoplasm surrounding the nucleus. The glial nucleus and the mitochondrion (*M*) were used as landmarks in the reconstruction to align neighboring sections. Magnification: $\times 31,000$. *B*, A neighboring section through the same varicosity at higher magnification ($\times 59,000$) to show details of the active zone (*arrow*).

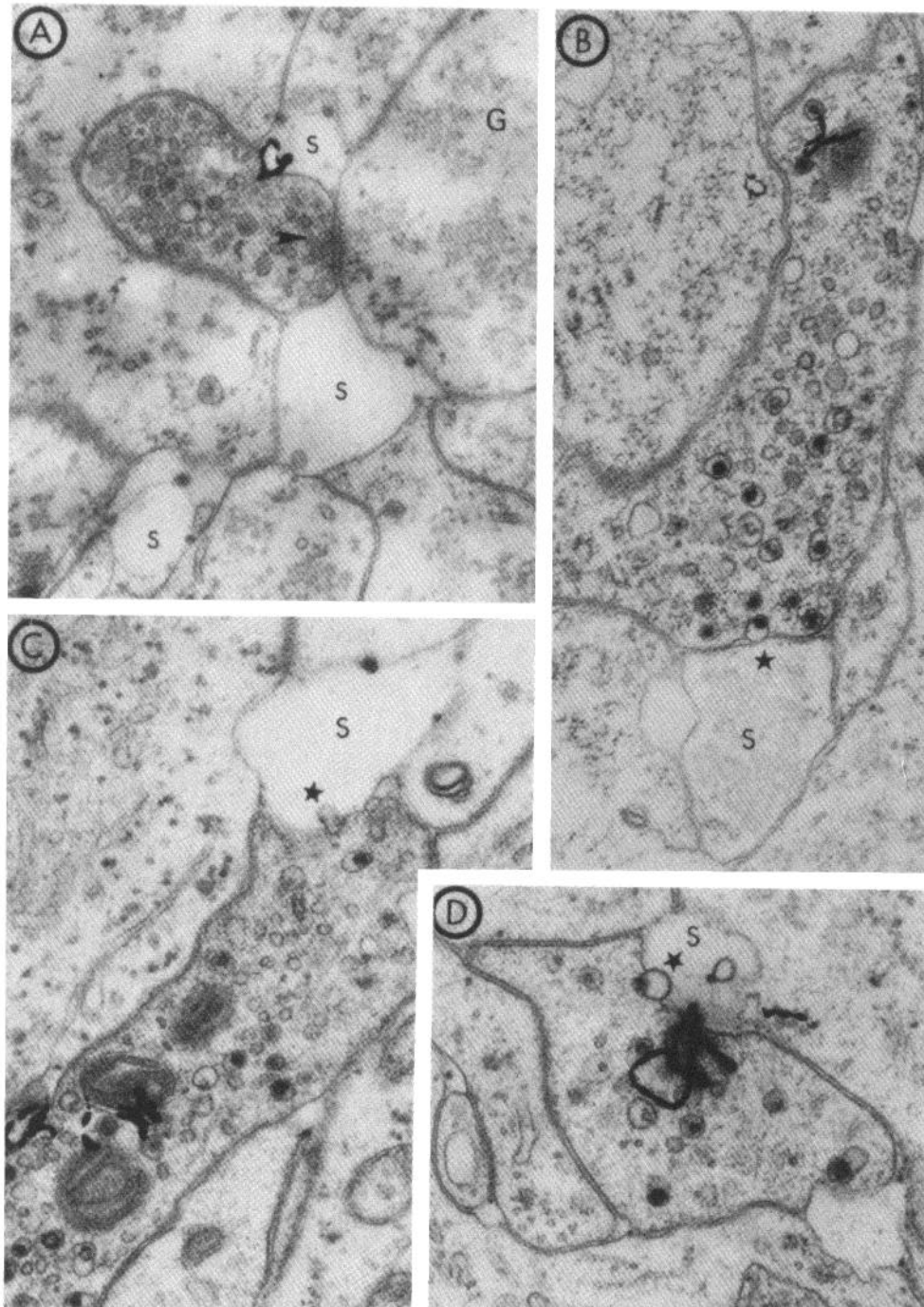


Figure 8. Association of GCN's varicosities in the buccal ganglion with a system of intraganglionic sinuses. *A*, A varicosity synapsing (*arrowhead*) on a glial cell (*G*) of the clear type within the glial capsule of the ganglion. Glial cells can be recognized by their accumulations of glycogen. The active zone appears to be surrounded by intercellular lacunae (*S*) that form a system of sinuses most prominent in the glial capsule but which also extends through the cell body layer to the neuropil at the core of the ganglion. Magnification: $\times 33,000$. In the other two regions of the ganglion, varicosities of GCN also are associated almost invariably with intercellular spaces. A few of GCN's varicosities show dense core vesicles interacting with membrane in regions lacking active zones (*stars*). *B*, A varicosity in the cell body layer; *C*, in the neuropil; *D*, another varicosity in the cell body layer. Magnifications: $\times 39,000$.

the abdominal ganglion treated with special stains revealed dense projections arranged in precise hexagonal grids over at least portions of the presynaptic area of unidentified *Aplysia* terminals. Unfortunately, it would

be quite difficult to use radioautography to identify membrane-delimited profiles together with these staining procedures because they give clearest results without osmication when membranes are unstained.

TABLE II

Comparison of the varicosities from two identified *Aplysia* neurons

Parameter	Sensory Neuron ^a	GCN
Surface area of varicosity (μm^2)	10.8	10.3-19.9
Area of active zone (μm^2)	0.17	0.12-1.25
Area of active zone/surface area of varicosity (%)	1.5	1.6-12.7
Vesicles associated with active zone (No./ μm^2)	100 ^b	15-94

^a Data from Bailey et al., 1979.

^b This value is likely to be overestimated. Vesicles within 50 nm of the active zone were counted in each of the serial sections (see Fig. 4), summed, and divided by the area of the zone. Bailey et al. (1979) found that 22% of vesicles appear in two adjacent sections, but we did not correct for this error.

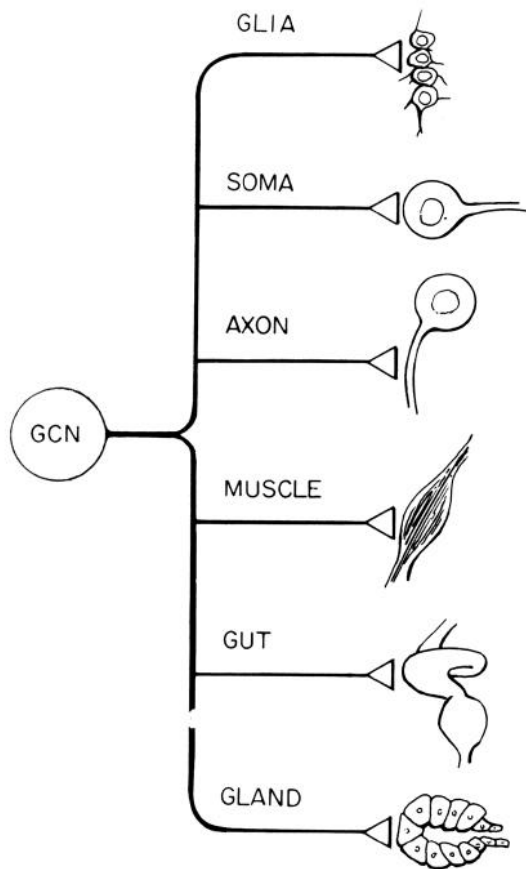


Figure 9. Diagram illustrating the variety of postsynaptic targets of the giant cerebral neuron. Synaptic contacts on glia and neurons have been described in this paper. See Weiss et al. (1978) for contacts on muscle. The terminals of GCN on the accessory radula closer muscle of the buccal mass have been identified only tentatively so far by radioautography after incubation of the isolated muscle in [³H]serotonin (L. J. Shkolnik, K. R. Weiss, and I. Kupfermann, unpublished experiments). There are no morphological studies on the synapses in gut and salivary gland; for electroanatomical evidence, see the work of Weiss and Kupfermann (1976) and Gelperin (1981).

Assuming that a hexagonal arrangement is the fundamental grid unit of active zones in *Aplysia*, Bailey et al. (1981b) showed that $0.06 \mu\text{m}^2$ would be the smallest

surface area possible for a synaptic contact; GCN's active zone then would be constructed of three of these units. Possibly suggesting that smaller synaptic grids are more effective than larger ones, Bailey et al. (1979) found that the number of vesicles per unit area of active zone is related inversely to the area of the active zone. The value of vesicle density that we found for GCN's active zone (Table II) nicely supports this observation.

Do the zones of apposition function as synapses? As originally proposed by Sherrington (1897), the term *synapse* clearly denoted a *functional* interaction between pre- and postsynaptic elements; his view of synaptic structure was an extensive area of contact between cells. Electron microscopic studies have provided morphological criteria for recognizing discrete regions of apposition that are likely to be sites where neurotransmitter is released. Later usage of the term *synapse* has accepted an anatomical connotation, and it is in this sense that we have used the term. The structures that we have observed meet the usual morphological criteria for a synaptic contact. In many other nervous systems that have been studied adequately, this kind of morphological contact has proven to be functional, and we presume that GCN's appositions also can be shown to be sites where transmitter is released. It should be noted, however, that release in the absence of morphologically identifiable synapses is believed to occur in autonomic adrenergic neurons and during development of the ciliary ganglia of the chick (Landmesser and Pilar, 1972).

Heterogeneity of GCN's postsynaptic targets. The widespread distribution of GCN's terminals indicates that this neuron innervates an extraordinarily broad postsynaptic field (Fig. 9). The transfer of information at synapses of a neuron is likely to depend not only on the specific transmitter substance released but also on the character and distribution of the postsynaptic receptor and its geometrical relationship to the presynaptic release site. For example, L10, a cholinergic interneuron of the abdominal ganglion, mediates excitation at some of its synapses, inhibition at others, and conjoint excitation and inhibition at still other synapses using the same transmitter substance, acetylcholine (Kandel et al., 1967; Wachtel and Kandel, 1967). GCN, like cell L10, is a multi-action cell which mediates different synaptic actions via its different branches to muscle, gut, and salivary gland (Gerschenfeld and Paupardin-Tritsch, 1974a, b; Weiss and Kupfermann, 1976). Electrophysiological studies have shown that the neuron has an extensive central distribution in the buccal ganglia which contain the cholinergic motoneurons that directly control the muscles involved in feeding (Cohen et al., 1978). Within the buccal ganglion, one of four distinct receptors to serotonin governs the production of synaptic potentials which are generated through specific changes in the postsynaptic membrane conductances of Na^+ and K^+ (Gerschenfeld, 1973; Gerschenfeld and Paupardin-Tritsch, 1974b). The GCNs also make extensive peripheral contacts in buccal musculature where they potentiate contraction through stimulation of a serotonin-sensitive adenylate cyclase (Weiss et al., 1978, 1979). Rather than initiating behavior directly, this modulatory function of GCN serves to enhance ongoing activity of muscle and moto-

neurons. Stimulation of cyclic AMP (adenosine 3':5'-monophosphate) in muscle is presumably under the control of peripheral terminals of GCN (L. J. Shkolnik, K. R. Weiss, and I. Kupfermann, unpublished experiments). Thus, some synapses of GCN function directly to excite or inhibit follower neurons through rapid alterations in postsynaptic conductance mechanisms and can be regarded as conventional fast synapses. Other endings of GCN have been shown to be modulatory in function, slowly regulating ongoing activity of other synapses.

Possible functional correlates of the serotonergic contacts. It would be interesting to determine whether the different morphological appositions that we have described correspond to synapses and to correlate the anatomical placement of GCN's synapses in the buccal ganglion with their functional properties. Specifically, it would be important to ascertain how the novel axosomatic appositions that are extremely rare in invertebrates differ functionally from the common axoaxonic synapses. The invaginating axosomatic varicosities often appear to touch the endoplasmic reticulum and penetrate almost to the nucleus. Do they exert any control over the synthesis of proteins or regulate the genome?

Similarly, it will be important to explore the contacts that GCN makes on glial cells. These contacts might change glial permeability to extracellular K^+ , modify transport of transmitters or other substances, or modulate the release of transmitters (see Colonnier et al., 1979; Güldner and Wolff, 1973; Soffer and Raine, 1980). Whatever its physiological role, input on glial cells might represent a novel synaptic action of this multi-targeted neuron that has not been suspected since its activity could not have been detected in the kinds of electrophysiological recordings from neurons that have been carried out so far.

It should be pointed out, however, that the glial cells might not be endowed with receptors for serotonin or any other transmitter substance that GCN might release. Thus, dissociated sympathetic neurons of newborn rats can release norepinephrine at autapses but are not sensitive to it because the neurons lack adrenergic receptors (Landis, 1976). These "presynaptic" sites of apposition without requisite "postsynaptic" receptors cannot be regarded as Sherringtonian synapses.

Even if a postsynaptic receptor is absent, GCN's contacts on glia might serve some important physiological function. Appositions with the morphological characteristics of synapses onto glia are not unique to invertebrates. They are uncommon in mature vertebrates but do occur in development (Henrikson and Vaughn, 1974; Grainger et al., 1968) and during regeneration (Singer et al., 1979; Soffer and Raine, 1980). In uninjured mature vertebrates, synaptic contacts on glial cells have been described in highly vascularized regions of the central nervous system that are neurosecretory or that lack the blood-brain barrier (Broadwell and Brightman, 1976); these include the medial vascular prechiasmatic gland, the median eminence, the neurohypophysis, the subcommissural organ, and ependymal cells within the ventricles (see Zimmerman, 1972; Güldner and Wolff, 1973; Tweedle and Hatton, 1980).

Because we found that all of the appositions of GCN

on glial cells neighbored sinuses, the most pertinent parallel in vertebrates is the extensive system of serotonergic axons that originate from dorsal and medial raphe neurons and which form supra- and subependymal plexuses in the walls of the ventricles, in the arachnoid sheath around major cerebral blood vessels, and in the pia over the spinal cord (see Aghajanian and Gallagher, 1975; Chan-Palay, 1975, 1976). These vertebrate neurons belong to the reticular activating system, a part of whose behavioral role is similar to that of GCN and homologous serotonergic neurons of other opisthobranch molluscs that function in the arousal and maintenance of feeding (Weiss et al., 1978; Gelperin and Jacobs, 1981). The proximity of the axoglial contacts to the system of ganglionic sinuses, which may be a neurohemal organ, suggests that the appositions on glia might have some general humoral function. Alternatively, the postsynaptic glial cells may serve to autoregulate specifically the microenvironment of GCN's terminals, perhaps by controlling the local ionic composition (see Kuffler and Nicholls, 1966; Tweedle and Hatton, 1980). Whatever their function may turn out to be, it is attractive to think that these junctions on glial cells are *syntelic* (working toward the same end; Greek: *syn*, together; *telos*, end), a term which we should like to introduce to signify that all of the synapses of a single neuron, no matter how diverse in structure or postsynaptic target, act together toward a common behavioral goal.

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