EFFECTS OF DEAFFERENTATION OF THREE
IDENTIFIED NEURONS IN THE LAST ABDOMINAL
GANGLION OF THE COCKROACH,
Periplaneta americana (Orthoptera)

A Dissertation for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
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This is to certify that the

thesis entitled

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presented by

Virginia M. Tipton

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ABSTRACT

EFFECTS OF DEAFFERENTATION OF THREE IDENTIFIED NEURONS IN THE LAST ABDOMINAL GANGLION OF THE COCKROACH, *Periplaneta americana* (Orthoptera)

By

Virginia M. Tipton

The present study was initiated to determine the morphological transneuronal effects of removal of afferent monosynaptic input from sensory neurons upon identified interneurons with which they make synaptic connections. Comparisons were made between normal and cercectomized adult male cockroaches and normal and cercectomized male seventh or eighth instar nymphs. Deafferentation was achieved by surgical removal of both anal cerci. The sensory neurons in the cerci provide the major presynaptic input to the interneurons of the last abdominal ganglion (A6). Three A6 neurons and their axons were consistently identifiable and were designated as interneurons I, II, and III.

Experimental animals were examined at 10 hours, 3 days, 8 days, one month, 3 months, and 5 months following cercectomy. Control animals had intact cerci. Somata were examined for changes in distribution of cytoplasmic RNA using autoradiography and pyronin Y staining. Ultrastructural qualitative comparisons of the somata and their axons were also made. Quantitative light microscopic investigations included measurements on 400x drawings of somata, nuclei, and nucleoli.

For electron microscopy, circumferences of axons in the connectives, 0.1-0.2 mm from ganglion A6, were measured on 8000x electron micrographs. The number of mitochondria within these axons was also counted on the same micrographs. Mitochondria and lysosomes of the somata were counted on 8000x electron micrographs within an area of 71.5 $cm^2$. For statistical analysis, each animal was considered a sample.

The results indicated that the morphology of the somata and axons of interneurons I, II, and III of both the nymph and adult cockroach was unaffected by massive removal of their sensory input. No qualitative or quantitative changes were seen in the parameters investigated.

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Virginia M. Tipton

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TABLE OF CONTENTS

# CHAPTER I

# INTRODUCTION

The specific purpose of this study was to determine the morphological transneuronal effects of removal of afferent monosynaptic input from sensory neurons upon identified interneurons with which they make synaptic connections. Prior work has established the functional and morphological interdependency of neurons. When connections between neurons are broken, morphological and functional abnormalities have been observed in the deprived, post-synaptic neurons. Most studies conducted thus far dealing with deafferentation, or surgical removal of sensory input, have involved projection pathways in the vertebrate nervous system. The morphological effects reported in these studies varied in intensity but generally indicated some degree of degenerative changes in the deafferented neurons (Cowan, 1970; Globus, 1975). Cowan (1970) recognized several levels of transneuronal degeneration: primary and secondary anterograde transneuronal degeneration and primary and secondary retrograde transneuronal degeneration. The author's research is concerned only with primary anterograde transneuronal degeneration, i.e., atrophy or degeneration at the first synapse due to deafferentation. For example, in the mammalian visual system, degeneration occurs in the lateral geniculate body following interruption of the optic nerve.

Jones and Thomas (1962) performed olfactory bulb resections on adult rats at an average age of 44 days. In Golgi stained preparations, they observed a decrease in the number of dendritic branches of the deafferented neurons. Matthews and Powell (1962) surgically removed the sensory input to the olfactory bulb in young rabbits aged 5-8 weeks. The animals were sacrificed after 6-200 days and sections of the olfactory bulb were stained either with thionin or by the Bodian method. All of the changes that occurred began within one month of the operation. Severe shrinkage of the layers of the bulb occurred with atrophy of the somata and dendrites.

Trumpy (1971) was also interested in the problem of degenerative changes in mature versus immature nervous systems. For his study he chose cats from 7 to 89 days of age. They were sacrificed from 24 hours to 14 days after deafferentation of the pontine nuclei by mesencephalic lesions. In the youngest kittens from 7-17 days of age, many neurons were degenerating at 36 hours and by 48 hours there was already some cell loss. Within 5 days most of the neurons had disintegrated. At first the nuclear chromatin clumped. This was followed by karyorhexis. The cells exhibited decreased basophilia and size reduction. Vacuolization of the neuropil appeared at 36 hours and persisted through the fifth postoperative day. By the fourteenth day, the pons was markedly shrunken. Animals older than 47 days showed only slight changes. Fourteen days after deafferentation, the neurons showed a "faint size decrease and basophilia." There were no changes in the neuropil. In cats from 21-42 days of age, there was some cell loss but not as much as in the very young animals. Vacuolization of the

neuropil was slight and a moderate shrinkage of the pons was observed. Trumpy also looked at deafferentation of the nuclei of the dorsal columns in 8-13 day old cats. Reactions in the external cuneate nucleus were identical to the pons, but after 14 days there was only a slight reduction in neuron size with no cell loss in the gracile and cuneate nuclei. He concluded that adverse transneuronal changes are the most pronounced in young animals that are still developing since all of the nuclei except the cuneate and gracile project to the cerebellar cortex which is the most immature part of the brain at birth. The cuneate and gracile nuclei probably had already passed the early, more sensitive stages of development. Rustioni and Sotelo (1974) reported the presence of post-synaptic specialization in neurons of the gracile nucleus of adult cats 6 months after deafferentation. These dendrites appeared to be normal as seen in their electron micrographs. Loewy (1972) also found cell shrinkage in kittens in deafferented neurons of Clarke's nucleus, but found no significant change in the size of deafferented neurons of adults.

A morphological study of transneuronal degeneration using electron microscopy was conducted by Pinching and Powell (1971). Unilateral destruction of the olfactory mucosa was performed on both rabbits and rats aged 5-7 weeks with sacrifices made from 10-90 days for rabbits and 10-250 days for rats. A pair of animals was taken at each sacrifice. Ultrastructural examination of degenerating neurons typically revealed increased electron density and shrinkage of the somata. It appeared to the authors that shrinkage of the cell body concentrated the protoplasmic contents to give the electron dense

appearance. The nuclear envelope was often vacuolated between the pores and nuclear contents lost distinction. In the cytoplasm there seemed to be a greater proportion of free ribosomes and fewer rosettes. Mitochondria, endoplasmic reticulum and the Golgi complex had a tendency to be swollen or vacuolated. The cytoplasm also appeared vacuolated. The same kind of degenerative changes was also noted in the dendrites in addition to dense packing and altered appearence of the neurotubules. Lysosomes seemed to be a little more common in the dendrites although this was not noted in the somata. The axons of the affected cells also showed a concentration of cytoplasmic contents and dense packing of tubules and filaments. Free ribosomes were common and mitochondria were swollen and disrupted. Occasionally lysosomes and large vacuoles were found. In severely affected axons, glycogen-like particles were present. There was shrinkage of the axons and their terminals with crowding and loss of distinction of the synaptic vesicles. Maximum degenerative changes occurred in the rabbits by the twenty-fourth postoperative days whereas degenerative changes did not begin in the rat until around day 200. The degenerative changes, however, were similar in all respects once they did occur in both species.

Other degenerative changes have been noted at the ultrastructural level of observation. Ghetti, et al. (1972) using adult rhesus monkeys observed degenerative changes in the lateral geniculate nucleus after eye enucleation. In the period from 4-7 days after deafferentation, degenerating neurons demonstrated a reduction in the number of mitochondria and infolding of the nuclei. Other changes in mitochondria,

nuclei, and endoplasmic reticulum (ER) were similar to those seen by Pinching and Powell except that in the monkey the ER seemed to lose its ribosomes. The rough ER of mice and rabbits seemed unchanged. At later times (10-36 days), proliferation of microtubules began and was widespread by 170 days at which time elongated and/or branching mitochondria were first noted. Also at this time, the rough ER seemed to have been "partially replenished" with ribosomes. The extreme electron density of cells seen by Pinching and Powell was not common. Two types of degenerating dendrites occurred, one type was similar to that observed by Pinching and Powell, that of electron density. The other type, called "watery" dendrites, appeared swollen, electron-lucent, and contained a floccular material and very few mitochondria. Jean-Baptiste and Morest (1975) noted proliferation of neurofilaments and depletion of vesicles and mitochondria in synaptic endings of deafferented trapezoid bodies of adult cats. Other than increased condensation of nuclear chromatin, no other cellular changes were obvious in electron micrographs.

The examples cited above are representative examples of the many studies that characterize transneuronal degenerative changes and indicate some of the problems involved. The reader is referred to the table in the review article of Globus (1975) which summarizes the species, age of the animals, sacrifice times, and results of deafferentation. There are very few reports of no significant reactive changes although as Cowan (1970) points out, "the effects of deafferentation vary inversely with the age of the animal at the time the deafferentation is carried out." That is, transneuronal changes are much more

severe in young animals. Comparisons of transneuronal degeneration are complicated by the use of different species of animals. A contributing factor to the complication might be the existence of different initial maturity levels among the species. Another problem using vertebrates is that their nervous system is so complex that identification of single neurons is virtually impossible. Therefore, populations of neurons must be examined, and there may be more than one type of neuron within the population. Loewy (1970, 1972) has already mentioned this as a source of possible error.

The invertebrate nervous system is made up of ganglia which consist of a ring of neuron cell bodies (cortex) around a central core of nerve processes (neuropil). The cell bodies, or somata, are usually monopolar and receive no synaptic connections. Since invertebrate nervous systems are relatively simple and offer the advantage of identification of individual neurons with newly developed dye techniques, (Stretton and Kravitz, 1968; Pitman et al., 1972), more research is now being conducted using invertebrates.

Studies of the relationship of the abdominal cerci and giant fibers began 39 years ago with the work of Pumphrey & Rawdon-Smith (1937) using electrophysiological techniques in which they discovered that stimulation of the cerci evoked responses in large axons (25-60μm in diameter) in the connectives which they called "giant fibers". They recorded activity from different points on the connectives which pass between the six abdominal and three thoracic ganglia to the sub-oesophageal ganglion. They concluded that there are synaptic connections between the abdominal sensory appendages,

the cerci and the giant fibers, and that the giant fibers were continuous from the last abdominal ganglion (A6) to the brain. Tactile or vibratory stimulation of the cerci cause a rapid escape response in the cockroach. Roeder (1948) and Callec & Boistel (1965) also suggested that the giant fibers ascend the nerve cord without synapses but that they terminate at the metathoracic ganglion (T3). Farley & Milburn (1969) and Harris & Smyth (1971) reported that the giant fibers pass through T3 and do not end there. Hess (1958b) showed degeneration following axotomy of the giant fibers to occur only up to T3. The somata of the giant fibers were all found to be located in A6 (Roeder, 1948; Farley & Milburn, 1969). The individual cockroach interneuron somata of A6 have only been discussed by Harris & Smyth (1971) and Tweedle et al., (1973). A discussion of them will be presented in "Materials & Methods" of this paper.

The only sensory inputs found so far to the giant fibers are from the cerci via the cercal nerves. Milburn & Bentley (1971), however, have suggested that there may be other input from intraganglionic neurons. It had been previously thought that the giant fibers directly drove the motor axons involved in the escape reaction. However, the average startle time was too long to be explained by direct synaptic connections. Dagan & Parnas (1970) and Parnas & Dagan (1971) finally demonstrated that the giant fibers could not excite leg motor neurons but could excite metathoracic interneurons. They also found smaller axons, activated by stimulation from the cercal nerves, that carried information directly to leg motor neurons. Parnas & Dagan (1971) also discovered that leg movement stimulated by other neurons could

be inhibited by stimulation of the giant fibers. They suggested from their results that high-velocity afferent impulses of the giants served to "clear-all-stations" by inhibiting all on-going activity. The impulses which reached the brain, were "interpreted, and integrated with other sensory input," and the appropriate responses were elicited. If there were direct synaptic activity between the giant fibers and leg motor neurons, a cockroach grooming its cerci would excite the giant fibers and elicit escape responses. The cockroach thus would continuously be trying to get away from itself. Parnas and Dagan (1971) have suggested the following scheme for inhibition of the escape response during grooming. The cerci activate the small and giant axons. The impulses of the giant axons travel faster because of their larger diameter, reach the brain which, based upon all sensory input, "interprets that there is no danger." The brain might activate the giant fibers which transmit the impulse back down to the thoracic interneurons which inhibit the excitatory slow-travelling small axons that would normally activate the leg motor neurons. The timing of the impulse travel and synaptic delays of all systems involved seem to support this theory of suppression of evasive behavior by the brain (Parnas and Dagan, 1971).

Thus far, very few studies have been done on the primary anterograde transneuronal effects of deafferentation in invertebrate nervous systems. Almost all of these are on animals within the same order, Orthoptera, in the class, Insecta plus one study on crayfish in the class, Crustacea.

In a study started in 1971 and continued in 1974 by Edwards & Palka (Edwards & Palka, 1971; Palka and Edwards, 1974), first instar nymphs of the house cricket, Acheta domesticus, were used. Paired posterior abdominal sensory appendages, the cerci, provide the major sensory input to the large interneurons of the last abdominal ganglion (Edwards & Palka, 1974). The cerci were either unilaterally or bilaterally amputated in newly hatched and subsequently removed each time they regenerated upon molting until the seventh instar or adult stage of development. When both cerci and subsequent regenerates were removed up to the sixth instar and then allowed to regenerate until adulthood, morphology of the cercal nerve and terminal ganglion were normal except for a reduction in neuropil volume in the ganglion. Electrophysiological responses, measured on two giant axons of large interneurons identified by Murphey (1973) and Edwards & Palka (1974) located in the last abdominal ganglion, were indistinguishable from those of control adults whose cerci had remained intact throughout development. With only one cercus throughout development to the adult stage, the volume of the neuropil was reduced by 30% on the deprived side, and no change was noted in the somata of the giant interneurons or their axons. Contralateral projections of cercal axons were found (this rarely occurs in normal animals). Electrophysiological responses on the intact side were normal but responses on the contralateral side were 10-20 times greater than normal. Normal responses in control animals were almost totally ipsilateral. Animals were also deprived of cerci for six instars and then allowed to regenerate only one cercus for the rest of their development.

These animals showed similar changes but to a lesser degree than those deprived of one cercus throughout development. Despite deprivation of their dominant sensory input, the somata and axons of the giant interneurons remained unchanged. According to Murphey (1973) "surprisingly normal dendritic fields" were found in adult cricket giant interneurons deprived of sensory input from the ipsilateral cercus at hatching.

Tweedle et al., (1973) studied the effects of deafferentation in adult cockroaches. They also cut off the anal cerci thus removing the major presynaptic input to the giant interneurons (Pumphrey & Rawdon-Smith, 1937; Roeder, 1948; Callec & Boistel, 1965). They reported the paired cells they examined probably correspond to giant fiber I of Harris and Smyth (1971). Cobalt dye was injected into these identified neurons and allowed to fill the dendrites and axons. They examined whole-mount preparations and found no observable changes in the form of the dendritic tree after 7 weeks of removal of the cerci.

Murphey and co-workers (Murphey et al., 1973; Murphey et al., 1975) were the first to demonstrate transneuronal effects in invertebrate neurons. They examined four uniquely identified interneurons by iontophoresis of cobalt dye into these neurons. They performed unilateral and bilateral cercectomies on crickets at hatching, and removed regenerating cerci at each molt until adulthood. Measurements were made on the length of reliably identifiable branches of the dendritic tree of each of the neurons. There were no significant changes in the basic branching pattern of the dendritic tree.

However, there was a reduction in length of the deafferented dendrites in bilateral cercectomy. The tables in their paper show significant changes with unilateral cercectomy, but the results do not follow a consistent pattern. However it does demonstrate independence of paired neurons with different treatments within the same ganglion. Finally, they noted a reduction in neuropil and cortex volume.

Bentley (1975) used genetic mutants of crickets to obtain removal of neural input rather than lesion techniques. He isolated mutants that lacked the sensilla (but apparently had the sensory neurons) on the cerci that normally provide the presynaptic input to the identified giant interneurons of the last abdominal ganglion such as the MGI (Murphey, 1973). Electrical stimulation of the cercal nerve did not elicit post-synaptic potentials in the MGI's. These interneurons when filled with cobalt dye by axonal diffusion appeared withered and smaller than those of the wild type. Measurements of chosen cross-sectional areas of the dendritic tree showed about a four-fold difference in size between the larger wild-types and smaller mutants. No significant differences between wild-type and mutant main dendrite shaft lengths, axon diameters, or general shape of the dendritic tree were detected. Bentley's study was the second to demonstrate changes in invertebrate neurons deprived of sensory input. The procedure was that of sensory deprivation rather than deafferentation by surgical means and the results were similar to those found in vertebrates (Globus, 1975) in that neurons suffer anatomically when their sensory input is removed. Bentley suggested "it may be that in the control of their development vertebrate and arthropod neurons have more in common than has previously been thought."

The RNA content of an injured neuron changes in concentration and distribution. Cohen & Jacklet (1965) observed in axotomized cockroach motor neurons dense "rings" of RNA surrounding the nucleus. These "perinuclear rings" occurred as early as 12 hours and reached a maximum at 2-3 days following axotomy. Cohen (1967) provided autoradiographic evidence of newly synthesized RNA in cockroach motor neurons of the metathoracic ganglion. He injected radioactive uridine into the region around a ganglion 24 hours after injury. Twenty-four hours later, he removed the ganglia, fixed them, and sectioned at 10μm. He observed high concentrations of perinuclear label. In a subsequent study, Young, Ashhurst, and Cohen (1970) looked for an ultrastructural manifestation of these perinuclear rings. Although they noted an increase in the number of ribosomes throughout the cytoplasm, they did not see any ribosomal distribution that would correspond to the perinuclear rings seen with the light microscope. They suggested the perinuclear ring might be an outpouring from the nucleus of messenger RNA (mRNA) which cannot be seen with the electron microscope. Byers' (1970) similar study on a different species of cockroach produced results in apparent conflict with those of Cohen (1967) and Young et al., (1970). Byers duplicated the autoradiographic procedure of Cohen (1967) and found at the most only 10-20% more silver grains near the nucleus in contrast to the high perinuclear concentration reported by him. However, when she injected radioactive uridine 1 to 6 weeks prior to instead of 24 hours after injury, she observed a pronounced concentration of label around the nucleus. She concluded that probably the labeled RNA had time to be incorporated

into ribosomes; therefore, the perinuclear ring represented a movement of Nissl substance toward the nucleus rather than newly synthesized RNA. Her ultrastructural analysis seemed to confirm this explanation for she observed on electron micrographs a greater concentration of Nissl substance around the nucleus of injured neurons than of normal neurons.

In vertebrates, the classical reaction to axotomy is chromatolysis, or dispersion of the Nissl bodies (clumps of rough ER) within the perikaryon. Generally, there is an increase in production of nucleolar and cytoplasmic RNA (Cragg, 1970, for review). Conversely, neurons deprived of synaptic input have exhibited a reduction in nuclear and cytoplasmic RNA. Trumpy (1971), using toluidine blue with RNAase controls in kittens, observed that transneuronally degenerating deafferented neurons in pontine nuclei showed less RNA than did controls in kittens. Rasch et al., (1961), using azure B, a stain that binds specifically to RNA, in retinal ganglion cells of cats dark-reared from birth, observed a significant reduction in nuclear, nucleolar, and cytoplasmic RNA. Comparable results were obtained for rats and chimpanzees dark-reared from birth.

Thus far, no electron microscopic studies have been conducted on the effects of deafferentation upon identified neurons in invertebrates. Since reports are now appearing indicating changes in invertebrates, (i.e. Bentley, 1975; Murphey et al., 1973; Murphey et al., 1975) one might suspect that perhaps ultrastructural changes might be seen. The present study was initiated out of this need for electron microscopic examination especially of the somata and axons deprived

of sensory input. The lack of quantification in many of the previous studies has also led me to apply quantitative procedures and statistical analysis wherever possible. The cockroach, Periplaneta americana, was chosen as the experimental animal as it was one of the two species used in all other studies in this particular area of interest. It was also decided to examine nymphs as well as adults since the only changes seen in deafferented invertebrate neurons thus far are those that occurred with deafferentation during development (Bentley, 1975; Murphey et al., 1975) and not in the adult (Wine, 1973; Tweedle et al., 1973).

# CHAPTER II

# MATERIALS AND METHODS

## General Procedures

Male adults and seventh or eighth instar nymphs of the cockroach, Periplaneta americana, used in these experiments were maintained on Purina Lab Chow pellets and water. Animals were anesthetized by placing them into a refrigerator until they were lethargic. Both anal cerci were completely cut off; and in the nymphs, recut when regeneration of the cerci occured. Control animals had cerci intact. The last abdominal ganglia (designated A6) and their connectives were examined at 10 hours, 3 days, 8 days, 1 month, 3 months and 5 months following cercectomy. The animals were dissected from the dorsal surface, and internal organs were removed exposing the ventral nerve cord. Removal of the last abdominal ganglion followed initial fixation in situ.

The connectives and nerves were cut as far from ganglion A6 as possible to prevent injury to the ganglion, and as much fat as possible was removed to show the outline of the ganglion for orientation in sectioning.

## Cytochemistry

Ganglia were fixed in Carnoy's Formula B (Humason, 1967) for 4 hours, dehydrated in a series of ethanol, cleared in toluene, and

embedded in Paraplast, Tissue Prep, or Tissuemat. Ten micron sections were stained with chloroform-extracted methyl green-pyronin Y (Kurnick, 1955). Control sections from each ganglion were treated 4 hours at 38°C with a sodium phosphate buffered solution of RNAase A and T at pH 7.2. (The RNAase was kindly provided by L.G. Robbins, Department of Zoology, Michigan State University.) Cells were examined for appearance and distribution of ribosomes indicated by the specific staining of pyronin Y for RNA. The methyl green was merely a counterstain for nuclei in this study.

Cobaltous Chloride Staining

A modification of the cobaltous chloride technique of Pitman et al., (1972) was used for identification of neurons and their processes. Freshly dissected A6 ganglia were immersed in an insect Ringer solution (Kerkut et al., 1969). The cut ends of the connectives were placed in 100mM cobaltous chloride. The two solutions were separated by a vaseline barrier through which the connectives extended and the cobaltous chloride allowed to diffuse into the connectives for 20-24 hours at 4°C. The ganglia were then immersed for 10 min. in 10ml of insect saline containing 0.05 to 0.1ml of concentrated ammonium sulfide solution. The ammonium sulfide reacts with the cobaltous chloride to form a black precipitate of cobalt sulfide within cells and neurites containing the cobaltous chloride. For electron microscopy the cobalt modification of Gillette and Pomeranz (1973) using 3,3'-diaminobenzidine tetrahydrochloride (DAB) was followed. The ganglia were then fixed and processed appropriately for light or electron microscopy.

Autoradiography

For autoradiography, 30μCi of uridine-5-$H^3$ in a volume of .05ml (obtained from New England Nuclear) was injected into the abdomen of each animal eleven days prior to the removal of the cerci. Water injected shams were used as controls. After 3 days, the labeled ganglia were removed, fixed, embedded, and sectioned as in the cytochemical studies. The sections on albumin-treated slides were coated with Kodak NTB-2 emulsion and stored for 2 months at 4°C in black, electrical taped slide boxes containing packets of Drierite. The slides were then developed in Kodak Dektol for 5 minutes and stained with either methyl green-pyronin Y or 1% methylene blue in 1% sodium borate. Cells were examined for distribution of radioactively labeled RNA.

Electron Microscopy

Both nymphs and adults were used in morphological examinations of three interneurons corresponding to I, II, III axons of Harris & Smyth (1971), their axons in the connectives, and the neuropil of the last abdominal ganglion (A6). Ganglia were fixed for 2 hours at 4°C in a modification of Karnovsky's (1965) fixative: 4% paraformaldehyde and 2.5% glutaraldehyde in 0.1M sodium phosphate buffer at pH 7.4. The final solution had a pH of 7.2 and a concentration of around 1730 milliosmols. Ganglia were rinsed in a cold 0.2M phosphate buffer at pH 7.2 containing 0.1M sucrose for one half hour followed by a rinse in fresh buffer for one half hour and were then postfixed for 1 hour in a 0.1M phosphate buffered 1% solution of osmium tetroxide in 0.1M sucrose. The ganglia were dehydrated in ethanol, infiltrated and embedded in either a mixture of Araldite 502, Epon 812, DDSA, DMP 30

(4:5:12:0.05) (M.E. Gardner, personal communication) or firm Spurr low-viscosity resin (Spurr, 1969). For light microscopy, thick sections (3μm) of the whole ganglia were placed on microscope slides, dried on a hot plate, immediately stained for about 5 seconds with 1% methylene blue containing 1% sodium borate and rinsed. These sections were used for identification orientation, location, and measurement of specific neurons. For electron microscopy, gold to silver (600-1000 Å) sections were taken on an LKB Ultratome III with a DuPont diamond knife or glass knives and double stained with aqueous saturated uranyl acetate for 1 hour and lead citrate for 5-10 minutes (Venable and Coggeshall, 1965). Uranyl acetate in 50% alcohol was used to stain sections embedded in Spurr's medium. Thin sections were taken 0.1-0.2mm from the entrance of the two connectives into the ganglion. The sections were examined with an Hitachi HU-11E transmission electron microscope at 75kv or a Phillips EM 201 at 60kv. In order to obtain prints of equal magnification, pictures of a crossed line carbon grating replica (54,800 lines/inch) were taken on each electron microscope.

Quantitative Procedure & Statistics

Cells from tissues processed for both light and electron microscopy were examined. Sections were projected with a Leitz Prado Universal projector onto white paper at 400x magnification. Outlines of the cells, nuclei and nucleoli were traced on the paper and cell and nuclear areas were measured with a Keuffel and Esser Co. planimeter. Cells with nucleoli were chosen for drawing. One 10μm paraffin section

and three 3μm plastic sections of the desired cells were drawn. The mean of the areas from the plastic sections was used for quantitative analysis. Since the fixatives and embedding media for light and electron microscopy affect the tissue differently, these specimens were analyzed separately. Nucleoli were measured with a Bausch & Lomb measuring magnifier #81-34-38. Nucleolar measurements used were obtained from the mean of the greatest and least diameters. For statistical purposes, each cell, nucleus, and nucleolus was considered a sample. To test for bias in using individual cells as samples, the mean of the areas of both cells of each of the three pairs and their nuclei in plastic sections were used so that each animal was considered a sample.

Circumferences of axons in the connectives, 0.1-0.2mm from the ganglion, were measured on 8000x electron micrographs with a Dietzgen plan measure #1719B. The number of mitochondria within these axons was also counted. Mitochondria and lysosomes of the soma were counted on 8000x electron micrographs within an area of 71.5cm$^2$. Since it was difficult to get both of each of the paired cells for electron microscopy, data from one of the pairs or means of the data from both cells of a pair were used from each animal, therefore each animal was considered a sample.

To control for possible significant growth differences in nymph interneurons, ganglia were taken from nymphs at about the sixth instar and ones of the next older instar. These ganglia were processed using the procedures described under "Cytochemistry" of this chapter and measured using the paraffin procedures described in this section of this chapter.

The Mann-Whitney U test for two independent samples and Kruskal-Wallis one-way analysis of variance for more than two independent samples were used for statistical analysis of the data described above.

Identification of Neurons

The term "identified neuron" applies to one so individually unique that it can be found consistently in each animal of a given species. Identification is based upon location and general appearance of the soma, dendritic tree and axon (Horridge, 1973). Cohen and Jacklet (1967) provided fairly detailed cell maps of the metathoracic ganglion of the cockroach. The cells were numbered and identified by a method employing injury response. When the axon of a neuron has been severed, within 12 hours a dense ring of RNA designated "perinuclear ring," determined using a pyronin-malachite green stain, appears around the nucleus (Cohen & Jacklet, 1965). This injury response provided a marker for identification of a particular neuron and also the muscle it innervated. Kennedy et al., (1969) made cell maps of crayfish ganglia and identified neurons and their processes by using the fluorescent dye technique of Stretton and Kravitz (1968). Mendenhall & Murphey (1974) described cricket giant interneurons of the terminal (A5) ganglion using backfilling of the axons with diffusing cobaltous chloride. This enabled the visualization of the axons, dendritic trees, and somata.

The present study also employed the filling of identified neurons by axonal delivery of cobalt. Whole mount preparations were used which had been treated with the dye, dehydrated in a graded series

of alcohols and either cleared in methyl salicylate or (without post-osmication) embedded in plastic. The preparations were examined with a Zeiss Photoscope II with photographs taken at a total magnification of 25.6 on 35mm negatives. Some of the plastic blocks were serially thick sectioned at 3μm. Looking at the whole mounts combined with the examination of serial sections allowed for identification of three neurons and their axons. For this study, the three cells and their axons have been designated I, II, and III (terminology of Harris and Smyth, 1971). Neurons I and III are located just anterior to nerve 4 (Guthrie & Tindall, 1968). They occur laterally as pairs in the cortex of the ganglion. Neuron I is dorsal to Neuron III (Plate 1a). Neuron II is the largest cell encountered posterior to nerve 4 (Plate 1b). Axon I is the most medial of the three in the connective, II lies next to I, and III is usually ventrolateral to II (Plate 2a). Neuron I seemed to correspond to cockroach fiber I of Harris and Smyth (1971) and to the cockroach A6 giant interneuron examined by Tweedle et al., (1973). There did seem to be a size discrepancy, however, between the small somata of giant I drawn by Harris and Smyth and the larger somata of this study and that of Tweedle et al., (1973). Mendenhall and Murphey (1974) admitted to possible homology (Based upon dendritic tree morphology) between their interneuron 8-1 of the cricket A5 ganglion (last abdominal ganglion) and fiber A of cockroach A6 (Milburn & Bentley, 1971). No somata were described by Milburn & Bentley. Upon comparison of neuron I from this study combined with that of Tweedle et al., (1973) with 8-1 of Mendenhall and Murphey, similarities were noted. It is then possible that cockroach neuron I (present study),

fiber I (Harris & Smyth, 1971), A6 neuron (Tweedle et al., 1973), and fiber A (Milburn & Bentley, 1971) are the same and are homologous with cricket interneuron 8-1 (Mendenhall & Murphey, 1974). This study's neuron II (Plates 1b & 2b) appeared to be the same as II of Harris & Smyth. Based upon axon location in the connective and soma position in the ganglion, there may be homology between neuron II and 8-1 of Mendenhall & Murphey. Neuron III (Plates 1a & 2b) may be the same as III of Harris and Smyth. The three small dorsal axons in connective A5-A6 (labeled 1, 2, 3 on Plate 2a) were not positively traced to identified somata but seemed to be axons of posterior paired neurons (labeled A & B on Plate 2b) which consistently filled with cobalt dye. If so, then there is possible homology between these and 9-2, 9-3, 10-2, or 10-3 of Mendenhall & Murphey.

# CHAPTER III

# RESULTS

## Cytochemistry

Since changes in the concentration and distribution of RNA in injured insect neurons (Cohen & Jacklet, 1965; Cohen, 1967; Young, Ashurst & Cohen, 1970; Byers, 1970) have been observed it was decided to examine cytoplasmic RNA content in this study. It was thought that perhaps changes might occur similar to the perinuclear rings described in axotomized cockroach neurons by Cohen & Jacklet (1965). Using the methyl green-pyronin Y stain with RNAase on control sections, perikarya of cells I, II and III of 30 adults (8 control, 7 three day cercectomized, 5 eight day cercectomized, 5 one month cercectomized, 5 three month cercectomized) were examined. No perinuclear rings were observed at any time in the three cells. Staining time was too varied for photometric analysis.

## Autoradiography

Autoradiography was performed to complement the results obtained from the cytochemical study. The autoradiographic study was designed to study movement of RNA within the cytoplasm, for the radioactive uridine would have been incorporated into cytoplasmic RNA before deafferentation. Only adult animals were used (5 three day cercectomized, 3 controls, 1 sham). Upon examination of the cells in question, it

was obvious that the silver grains were fairly equally distributed in the cytoplasm of the control and three day group (Plate 3). Only a small amount of background radioactivity was detected in the water injected sham and RNAase control slides. There was apparently no redistribution of cytoplasmic RNA after deafferentation of three days.

Electron Microscopy

The reader is referred to Plates 4-18 for this section.

A. Normal Morphology

1. Neuron Somata: No morphological differences in basic composition or structure were noted between the nymph and adult neurons nor between neurons I, II & III and so will be discussed as one.

Cells I, II, & III of this study seemed to be the "light" type described by Hess (1958a). Using the electron microscope, Hess described two neuronal cell types: "dark" and "light" cells and described a third "vacuolated" cell seen through the light microscope which this author found most frequently in the dorso-medial cortex. The three neurons of the present study were all "light" cells. Cells I, II and III were among the largest in the ganglion, 65-75μm in diameter with nuclei 25-35μm and nucleoli 4-7μm in diameter. All other large cells within this size range were also "light." The smaller neurons fit into the "dark" category. Hess observed the Nissl substance to be clumped in the lighter cells and more dispersed in the darker ones thus giving the impression of being darker. The results of this study were in agreement with that observation.

The nucleus was rarely seen to be spherical in shape. Rather, as seen with the light microscope, it is more like an oblate spheroid

with an indented side that seems to have no preferred orientation. Ultrastructural analysis reveals an irregular outline with many indentations (Plate 11). This probably allows a larger nuclear surface area which could then allow greater nuclear-cytoplasmic interaction, i.e., there could be more movement of ribosomal precursors and messenger RNA from the nucleus to the cytoplasm. Nuclear pores were seen to occur at about 120-160nm intervals. The nucleoplasm was a diffuse granular matrix containing one very compact nucleolus. At no time was more than one nucleolus per nucleus seen. Byers (1970) has observed one or two nucleoli in another species of cockroach, Diploptera punctata. The majority of the nucleolus seemed to be composed of both granules and short fibrils typical of the pars fibrosa. A pars granulosa was sometimes present. The nucleolus had holes which appear to contain the same material as the general nucleoplasm and were thus considered to be channels within the nucleolus. Another feature of the nucleus was the occurrence of an inclusion about one-half to two-thirds the size of the nucleolus. It appeared as a lightly staining area containing mostly fibrous material (Plate 9). The nature of the chemical composition of this area is unknown, but, based on the staining properties and fibrous nature, might be protein in nature.

On the periphery of each neuron cell body was the trophospongium consisting of a complex of fine cytoplasmic projections of the surrounding glial cells into the soma of the neuron (Plate 6). The trophospongium is presumed to be nutritive in nature (Bullock & Horridge, 1965).

Mitochondria were abundant and distributed equally throughout the cytoplasm. They varied in thickness (from 60-600nm) and length. The length was difficult to assess because they were long enough and curved enough to be only partially present in the thin sections necessary for electron microscopy. The mitochondria frequently occurred in association with clumps of Nissl substance, Golgi, glycogen patches, nuclei, and large lysosomes (Plates 6, 7, 10, and 12). Golgi complexes also seemed to be fairly randomly scattered in the cytoplasm with slightly greater numbers of the periphery (Plate 13). This observation, made on electron micrographs with a magnification of 8000x, was based on a comparison of the numbers of Golgi bodies occurring within 7.5μm of the plasma membrane. Byers (1970), also felt they were located mostly in the periphery of neurons 3L and 3R in the metathoracic ganglion of the cockroach, Diploptera punctata. The number of cisternae varied from 5-10 with slight dilations of one or two of these at the convex face. Small vesicles, 50-70nm in diameter, were found in association with the concave face. These vesicles were either empty or filled with a dense material. Lysosomes and multivesicular bodies were also found at the concave face (Plate 13). There seemed to be no preferred orientation of the Golgi (Plate 13).

The basophilic regions of the cytoplasm were irregularly shaped clumps of Nissl substance scattered throughout a rather electronlucent ground substance and also surrounding the nucleus (Plate 12). Byers (1970) considered them to be Nissl bodies comparable to those of vertebrate neurons. Cohen (1967) defines Nissl bodies as large cytoplasmic basophilic aggregates seen in the neuron soma with the

light microscope and Nissl substance as uniform cytoplasmic basophilia. The most widely held opinion seems to be that invertebrate neurons lack Nissl bodies for in sections viewed through the light microscope, no distinct regions are seen that could be called Nissl bodies. Byers believed this was due to the random distribution of these aggregates which would overlap in thick sections and thus be masked. However, there still seems to be a major difference in the vertebrate neuron Nissl and that of the cockroach. Most of the ribosomes of the majority of vertebrate neuron types is bound to the endoplasmic reticulum as rough ER. Most of the ribosomes are free in the cockroach neurons that have been studied (Byers, 1970, Hess, 1958a; Smith and Treherne, 1963; 1970; Young et al., 1970; the present study) and most other invertebrates (Cohen, 1970). The ribosomes of neurons I, II, & III occurred either singly or in clusters. There was very little rough or smooth endoplasmic reticulum. These clusters of ribosomes and smooth and rough ER were found in a fine, lightly staining matrix presumed to be protein. There were a few microtubules, some smooth ER and a small amount of floccular material in the electron lucent areas of the cytoplasm.

Large patches of glycogen (Plates 5 & 6) 2-6μm long and 1.5-3.5μm wide were located on the periphery and were especially abundant in the part of the soma directly adjacent to the link segment. Smaller patches and individual glycogen granules could be found toward the interior of the cell.

Three basic types of lysosomes were recognized. Primary lysosomes, 60-200nm in diameter, were scattered throughout the cytoplasm.

Multivesicular bodies, 200-400nm across, were found less frequently than primary lysosomes. Another type didn't seem to fit into any of the usual categories of primary lysosomes or secondary lysosomes, i.e., multivesicular bodies, autophagic vacuoles, or residual bodies. Historically, both these inclusions and Golgi bodies were called "lipochondria" by Baker in 1950, but later Golgi were eliminated from this category (Baker, 1963). It was discovered in cytochemical studies in locust and snail neurons that these lipochondria probably contained thiamine diphosphatase and acid phosphatase (Baker, 1963). The "lamellar bodies" in Blaberus craniifer pars intercerebralis neurons (Willey & Chapman, 1962), the lipid "colorless globules" in Helix aspersa cerebral ganglia neurons (Chou & Meek, 1958), the "lipochondria" in Periplaneta americana thoracic ganglia neurons (Hess, 1958a), the "lipochondria" of Melanoplus differentialis thoracic ganglia neurons (Lane, 1968), and the large unusual lysosomes of the present study all seemed to ultrastructurally resemble the "lipochondria" described by Baker. Lane (1968) offered further cytochemical analyses correlated with ultrastructural studies that these lipochondria, so common in vertebrate neurons, were, in fact, lysosomes which were described as structures 0.5-1.5μm in diameter bounded by a single membrane and containing many parallel membraneous lamellae. Similar lysosomal bodies were observed in the present study (Plate 4). Others were only partially filled with lamellae, the rest being either homogeneously dense or containing some globular inclusions which might be mitochondria (Plate 8). These lipochondrial lysosomes are frequently found in close association with large glycogen deposits (Plate 8).

They may be involved in rapid mitochondrial turnover. Mitochondria predominate over "lipochondria" around large glycogen deposits, but "lipochondria" are observed to occur more frequently in smaller deposits. Finally, "lipochondria" predominate around the very small deposits of glycogen. Pyruvate, a cytoplasmic metabolite of glycogen, is further metabolized in mitochondria to generate energy in the form of ATP. The close association of the lipochondrial lysosomes with the mitochondria and glycogen aggregates (Plate 6) plus the appearance of greater numbers of "lipochondria" with smaller, depleted glycogen reserves seems to indicate that these are secondary lysosomes digesting functionally exhausted mitochondria.

Lysosomes the same size as and with very similar ultrastructure as "lipochondria" have been found in pathologic vertebrate neurons. These "zebra-bodies" have been found in individuals with Hunter-Hurler syndrome, which is a metabolic disturbance involving lipids and muco-polysaccharides (Aleu et al., 1965). Lane (1968) reported several studies of vertebrate lysosomes with the ultrastructural features of lipochondrial lysosomes. Koenig (1969) reported the abundance of lysosomes in vertebrate neurons, but only noted lipochondrial-like lysosomes when neutral red, a vital dye, was incorporated causing "remarkable changes in their fine structure in brain...of rat." He also noted that this was accompanied by a 16-27 fold increase in their volume.

2. Normal axons (Plates 14 & 16): Axons I, II, and III were surrounded by a rather thick, laminated glial sheath. Unlike the myelin sheath of vertebrate axons, this glial sheath had thin

layers of cytoplasm between the membranes. Occasional small glial projections were seen extending into the axoplasm (Plate 15). Neurofilaments, neurotubules, and mitochondria were parallel to one another and oriented along the long axis of the axon. Their distribution within the axoplasm was otherwise random (Plates 16 & 17).

B. Deafferented Neurons

1. Somata: Qualitative examination of deafferented neurons of nymphs at 10 hours, 3 days, 8 days, and one month and adults at those same time periods plus 5 months revealed no observable degenerative changes. For example, there was no swelling, shrinking, or vacuolization of any of the membrane-bound cytoplasmic inclusions such as Golgi, ER, nuclei, and mitochondria. No darkening of the cytoplasm occurred, nor did the distribution or amount of cytoplasmic Nissl substance shift or seem to change in amount. I also looked for changes in orientation and general shape of the Golgi, blebbing of mitochondria and alterations in arrangement of the cristae. There were no obvious differences between control and experimental animals in either adults or nymphs. The trophospongium seemed to extend into the cytoplasm of the perikaryon as much in controls as experimentals (compare Plates 9 and 12). There was also no evidence of change in the nucleoplasm or nucleolus. Attempts were made to measure the size of the Golgi complex but because of their curved nature, it was extremely difficult to get reliable quantitative data. From these attempts however, plus general observation, there seemed to be no change in their size, number, or swelling of cisternae.

Since a displacement of the nucleus to an eccentric position had been observed within a week of injury to cockroach motor neurons (Jacklet & Cohen, 1967), I decided to look for a similar reaction in this study. Examination of the semi-thin sections taken for light microscopy revealed no shift in position of the nucleus except in 2 individuals. One nymph neuron II deafferented for one month and paried adult neurons I and one II deafferented from the same animal for one month had eccentric nuclei. Since these were the only such neurons of the 54 one month cercectomized neurons examined, the author did not consider this shift in position of the nucleus of these four cells to be attributable to deafferentation.

2. Deafferented Axons: No changes were observed in the axons following cercectomy. Hess (1958b, 1960) reported degenerative orthograde and retrograde changes in cockroach connective axons following axotomy in the form of clumping of the axoplasm and its inclusions, a general increase in electron density of the axon, very dense and enlarged mitochondria, and a striking increase in density and in the thickness of the layers of glial processes surrounding the degenerating axons. After a week, degenerating fibers had collapsed and disappeared. In a comparison to Hess' results, qualitative observations in this study of mitochondrial size, thickness and density of the periaxonal glial sheath and axoplasmic density revealed no observable changes. There was also no redistribution of mitochondria, microtubules, or microfilaments in the deafferented axons I, II or III. No significant difference was observed between control and experimental animals in invasion of the axoplasm by neuroglial processes,

nor was any reactive vacuolization of axoplasm or mitochondria observed (compare Plates 16 & 17).

3. Neuropil: Attempts at identification of dendrites of cells I, II, and III proved futile with the methods available at the time of this study. Single-cell injections of dye with microelectrodes will have to be used to positive identification of only one cell. Diffusion of the cobalt dye through the connectives always filled more than one cell. It was observed, however, that following the technique of Gillette and Pomeranz (1973) using DAB produced distortion in the filled neurites while the rest of the neuropil appeared fairly normal (Plate 17). The author also observed orthograde degeneration of the cercal fibers within the neuropil as early as 10 hours following cercectomy.

## Quantitative Results

The probability levels quoted in this section were obtained from the Mann-Whitney U test and Kruskal-Wallis one-way analysis of variance. No significant difference in size was observed in the control experiment run on nymphs of two successive age groups (Table 13); therefore, statistics based upon experimental nymphs should be valid.

With two exceptions, all of the parameters examined for quantitative analysis clearly showed no reactive change to deafferentation ($p>.1$) (Tables 1-15). Measurements of the surface area of the projected cells in nymphs and adults of all three cells processed both for paraffin and plastic embedding demonstrated a resistance to change in the size of the somata following deafferentation (Tables 1-4).

They neither shrank nor swelled. Nor was there any significant change in the circumference of axons of neurons I, II, and III. The two exceptions mentioned were found in nymph neuron II in the paraffin sections, (Table 1). Both the somata ($p=.01$) and the nuclei ($p=.05$) apparently increased in size when examined one month after deafferentation. The author believed this was probably not the case since one would expect, based upon results from other studies of deafferentation (see introduction), shrinkage rather than swelling. This doubt is strengthened by the fact that no changes were observed in the identical cell of the plastic sections ($p>.1$). Probably this was due to the small sample size rather than a true transneuronal reaction. Since loss of mitochondria has been demonstrated in transneuronally degenerating systems, the number of mitochondria in both the perikarya and axons was also determined. The results indicated no difference between normal and deafferented neurons ($p>.1$) (Tables 5-6, 9-10). There were no significant changes in nucleolar or axon sizes of any of the three cells examined in the nymphs and adults ($p>.1$) (Tables 7-8, 11-12). Quantitative analysis of lysosomes (Table 14-15) plus a qualitative overview revealed no obvious increase or decrease in number of lysosomes in the experimental animals.

CHAPTER IV

TABLES

Table 1. Ranking of Nymph Cell and Nucleus Area Measurements* (Paraffin Sections)

| CELL AREA | | | NUCLEUS AREA | | |
|---|---|---|---|---|---|
| Cell I | Cell II | Cell III | Nucleus I | Nucleus II | Nucleus III |
| C 1.3 | C 1.2 | C 1.3 | C 0.3 | C 0.3 | C 0.3 |
| C 1.3 | C 1.3 | C 1.8 | 1 0.4 | C 0.3 | C 0.4 |
| 1 1.3 | C 1.3 | C 1.8 | C 0.5 | 1 0.3 | 1 0.4 |
| C 1.4 | C 1.5 | 1 1.8 | C 0.5 | C 0.4 | 1 0.4 |
| 1 1.4 | 1 1.5 | C 1.9 | 1 0.5 | 1 0.4 | C 0.5 |
| 1 2.0 | 1 1.5 | 1 1.9 | 1 0.5 | 1 0.4 | C 0.5 |
| | 1 1.7 | | | 1 0.4 | |
| | 1 1.8 | | | 1 0.4 | |
| | 1 1.8 | | | 1 0.5 | |

C = control
1 = 1 month cercectomized
p >.1 cells I & II and nuclei I & II
p = .01 cell II
p = .05 nucleus II
*Measured in $cm^2$ on a 400x drawing.

Table 2. Ranking of Adult Cell and Nucleus Area Measurements*
(Paraffin Sections)

| CELL AREA | | | NUCLEUS AREA | | |
|---|---|---|---|---|---|
| Cell I | Cell II | Cell III | Nucleus I | Nucleus II | Nucleus III |
| C 1.3 | 1 1.1 | C 1.4 | C 0.2 | C 0.2 | 1 0.3 |
| C 1.4 | C 1.3 | 1 1.5 | C 0.3 | C 0.3 | C 0.4 |
| 1 1.4 | 1 1.3 | 1 1.5 | C 0.3 | C 0.3 | C 0.4 |
| 3 1.5 | C 1.4 | 1 1.6 | 3 0.3 | C 0.3 | C 0.4 |
| 1 1.6 | C 1.5 | C 1.7 | C 0.4 | C 0.3 | C 0.4 |
| 3 1.6 | C 1.5 | C 1.7 | C 0.4 | C 0.3 | 3 0.4 |
| 3 1.6 | 3 1.6 | C 1.8 | 3 0.4 | 1 0.3 | C 0.5 |
| C 1.7 | C 1.7 | 1 1.9 | 3 0.4 | 1 0.3 | C 0.5 |
| 1 1.7 | 3 1.7 | 3 1.9 | C 0.5 | 3 0.3 | C 0.5 |
| C 1.8 | 3 1.7 | 3 1.9 | 1 0.5 | 3 0.4 | 3 0.5 |
| 1 1.8 | C 1.8 | 3 2.0 | 1 0.5 | 3 0.4 | 3 0.5 |
| 3 1.8 | 1 1.8 | 3 2.0 | 1 0.5 | 3 0.4 | C 0.6 |
| 1 1.9 | 1 1.8 | 3 2.0 | 1 0.5 | C 0.5 | 1 0.6 |
| 1 1.9 | 1 1.8 | 1 2.1 | 3 0.5 | C 0.5 | 1 0.6 |
| 3 1.9 | 3 1.8 | C 2.2 | 3 0.5 | 1 0.5 | 3 0.6 |
| 3 1.9 | 3 1.8 | C 2.2 | 3 0.5 | 3 0.5 | 3 0.6 |
| C 2.0 | 3 1.8 | 3 2.2 | C 0.6 | 3 0.5 | 3 0.6 |
| C 2.0 | 1 1.9 | 3 2.2 | C 0.6 | 3 0.5 | 3 0.6 |
| C 2.0 | 3 1.9 | C 2.3 | 1 0.6 | 3 0.5 | C 0.7 |
| 3 2.0 | 1 2.0 | C 2.4 | 1 0.6 | 3 0.5 | 1 0.7 |
| 3 2.0 | 3 2.0 | C 2.6 | 3 0.6 | 1 0.6 | 1 0.7 |
| C 2.1 | 3 2.0 | 1 2.7 | 3 0.7 | 1 0.6 | |
| | C 2.1 | | | 3 0.6 | |
| | C 2.1 | | | C 0.7 | |
| | C 2.1 | | | | |
| | 3 2.1 | | | | |

C = control
1 = 1 month cercectomized
3 = 3 months cercectomized
p >.1
*Measured in $cm^2$ on a 400x drawing.

Table 3. Ranking of Nymph Cell and Nucleus Area Measurements*
(Plastic Sections)

| CELL AREA | | | NUCLEUS AREA | | |
|---|---|---|---|---|---|
| Cell I | Cell II | Cell III | Nucleus I | Nucleus II | Nucleus III |
| 1 2.42 | C 2.84 | 1 2.62 | 1 0.25 | 1 0.29 | 1 0.34 |
| C 2.67 | 1 2.99 | C 3.37 | C 0.32 | C 0.32 | 1 0.43 |
| 1 2.67 | C 3.02 | 1 3.54 | C 0.39 | 1 0.37 | 1 0.44 |
| C 2.72 | 1 3.07 | 1 3.65 | C 0.39 | C 0.4 | C 0.45 |
| 1 3.0 | 1 3.12 | 1 3.7 | 1 0.39 | 1 0.4 | C 0.47 |
| C 3.09 | 1 3.15 | 1 3.75 | 1 0.39 | 1 0.4 | C 0.47 |
| 1 3.27 | C 3.18 | C 4.12 | 1 0.4 | C 0.45 | 1 0.5 |
| 1 3.72 | C 3.18 | C 4.9 | 1 0.54 | 1 0.49 | 1 0.55 |

C = control
1 = 1 month cercectomized
$p > .1$
*Measured in $cm^2$ on a 400x drawing.

Table 4. Ranking of Adult Cell and Nucleus Area Measurements*
(Plastic Sections)

| CELL AREA | | | NUCLEUS AREA | | |
|---|---|---|---|---|---|
| Cell I | Cell II | Cell III | Nucleus I | Nucleus II | Nucleus III |
| 1 3.38 | 1 3.25 | 1 4.55 | C 0.39 | 1 0.32 | C 0.47 |
| 1 3.5 | C 3.3 | 1 4.72 | 1 0.39 | C 0.33 | 1 0.5 |
| C 4.04 | 1 3.77 | C 4.73 | C 0.42 | C 0.34 | 1 0.52 |
| C 4.15 | 1 4.07 | C 4.73 | 1 0.42 | 1 0.35 | C 0.53 |
| C 4.17 | C 4.29 | 1 4.92 | 1 0.43 | 1 0.42 | C 0.54 |
| 1 4.17 | C 4.7 | 1 5.03 | C 0.47 | C 0.44 | 1 0.63 |
| 1 4.39 | 1 5.02 | C 5.17 | 1 0.57 | 1 0.5 | 1 0.74 |
| C 5.18 | C 5.25 | C 5.65 | C 0.6 | C 0.55 | C 0.77 |
| C 6.37 | C 6.02 | C 6.1 | C 0.72 | C 0.55 | C 0.84 |

C = control
1 = 1 month cercectomized
$p > .1$
*Measured in $cm^2$ on a 400 x drawing.

Table 5. Ranking of Number of Mitochondria in Nymph Cells*

| Cell I | | Cell II | | Cell III | |
|---|---|---|---|---|---|
| C | 98 | 1 | 115.5 | C | 92 |
| 1 | 100.5 | 1 | 121.5 | C | 95.8 |
| C | 119 | 1 | 122 | 1 | 98.5 |
| 1 | 122.5 | C | 126 | 1 | 107 |
| 1 | 124 | C | 135.5 | 1 | 117.5 |
| 1 | 162 | C | 138.5 | 1 | 124 |
| C | 189 | 1 | 157 | C | 141 |
| | | 1 | 232 | C | 145.1 |

C = control
1 = 1 month cercectomized
$p > .1$
*Counted on an 8000x Electron Micrograph in Area 71.5$cm^2$.

Table 6. Ranking of Number of Mitochondria in Adult Cells*

| Cell I | | Cell II | | Cell III | |
|---|---|---|---|---|---|
| C | 48 | C | 66.5 | C | 74 |
| C | 82.5 | C | 80.5 | C | 82 |
| 1 | 87 | 1 | 86 | 1 | 90 |
| C | 91 | C | 92 | C | 91 |
| C | 107 | 1 | 97 | C | 99 |
| 1 | 113 | C | 106 | 1 | 102 |
| 1 | 122 | 1 | 139 | 1 | 177 |
| 1 | 146 | 1 | 142 | 1 | 144 |
| C | 147 | C | 147 | | |

C = control
1 = 1 month cercectomized
$p > .1$
* Counted on an 8000x Electron Micrograph in Area 71.5$cm^2$.

Table 7. Ranking of Diameters of Adult Nucleoli*
(Plastic Sections)

| Nucleolus I | Nucleolus II | Nucleolus III |
|---|---|---|
| C 1.97 | C 2.25 | C 2.45 |
| 1 2.35 | C 2.4 | C 2.55 |
| 1 2.45 | 1 2.5 | C 2.65 |
| C 2.5 | C 2.55 | C 2.7 |
| C 2.5 | C 2.55 | 1 2.7 |
| 1 2.5 | 1 2.6 | C 2.75 |
| C 2.55 | 1 2.6 | 1 2.75 |
| 1 2.55 | C 2.65 | 1 2.75 |
| C 2.6 | 1 2.75 | 1 2.8 |
| 1 2.6 | 1 2.75 | 1 2.9 |
| C 2.65 | C 2.85 | C 2.95 |
| 1 2.65 | 1 2.9 | 1 3.0 |
| C 2.7 | C 3.1 | C 3.05 |
| 1 2.9 | | 1 3.1 |

C = control
1 = 1 month cercectomized
$p > .1$
*Measured in mm on a 400x drawing.

Table 8. Ranking of Diameters of Nymph Nucleoli*
(Plastic Sections)

| Nucleolus I | | Nucleolus II | | Nucleolus III | |
|---|---|---|---|---|---|
| 1 | 1.65 | C | 2.1 | 1 | 2.05 |
| C | 1.9 | C | 2.15 | C | 2.1 |
| C | 1.95 | C | 2.2 | 1 | 2.15 |
| 1 | 1.95 | 1 | 2.3 | C | 2.4 |
| 1 | 1.95 | 1 | 2.35 | 1 | 2.4 |
| C | 2.05 | C | 2.4 | C | 2.45 |
| 1 | 2.05 | C | 2.4 | C | 2.45 |
| C | 2.15 | 1 | 2.4 | 1 | 2.55 |
| C | 2.15 | 1 | 2.4 | C | 2.6 |
| 1 | 2.2 | 1 | 2.45 | 1 | 2.6 |
| 1 | 2.2 | C | 2.5 | 1 | 2.75 |
| 1 | 2.4 | 1 | 2.5 | C | 2.8 |
| 1 | 2.5 | 1 | 2.5 | 1 | 2.85 |
| 1 | 2.55 | 1 | 2.75 | | |

C = control
1 = 1 minth cercectomized
p > .1 nucleoli I & II
p > .1 nucleolus II
*Measured in mm on an 400x drawing.

Table 9. Ranking of Number of Mitochondria in Adult Axons*

| Axon I | | Axon II | | Axon III | |
|---|---|---|---|---|---|
| C | 27 | C | 26 | C | 20 |
| 3 | 46.5 | 3 | 64 | 8 | 59 |
| 8 | 47 | 3 | 66 | 3 | 61.5 |
| 3 | 53 | 8 | 84 | 3 | 66.5 |
| 1 | 53.5 | 1 | 98 | 1 | 69.3 |
| 5 | 70 | 5 | 118 | 5 | 73 |
| 5 | 73 | 1 | 121 | 1 | 91 |
| 1 | 75 | C | 130 | 1 | 121 |
| C | 100 | 1 | 133 | C | 125 |
| 10 | 110 | C | 142 | C | 140 |
| 10 | 120 | 10 | 169 | 10 | 161 |
| C | 123 | 10 | 199 | 5 | 191 |
| C | 132 | C | 271.5 | 10 | 192 |
| 1 | 136 | | | C | 264 |
| C | 195 | | | | |

C = control
10 = 10 hours cercectomized
3 = 3 days cercectomized
8 = 8 days cercectomized
1 = 1 month cercectomized
5 = 5 months cercectomized
$p > .1$
*Counted on an 8000x Electron Micrograph.

Table 10. Ranking of Number of Mitochondria in Nymph Axons*

| Axon I | | Axon II | | Axon III | |
|---|---|---|---|---|---|
| 1 | 19 | 1 | 39.5 | 1 | 44.7 |
| 1 | 29.5 | 3 | 44 | C | 50 |
| 10 | 30 | 8 | 46 | 3 | 53 |
| 8 | 30 | 1 | 55 | C | 55 |
| 3 | 34 | 1 | 55.3 | 10 | 55 |
| 1 | 35.5 | 10 | 57 | 8 | 55 |
| 8 | 36 | 3 | 65 | 1 | 55.5 |
| 8 | 36.5 | 8 | 66 | 8 | 62 |
| 1 | 38 | 8 | 71 | 3 | 64 |
| 10 | 40 | C | 74 | 10 | 66.5 |
| 3 | 41 | 3 | 78 | 1 | 67 |
| C | 53 | 1 | 80 | 1 | 68 |
| 1 | 53.5 | 1 | 84 | 1 | 71 |
| 3 | 64.5 | 10 | 93 | 3 | 76 |

C = control
10 = 10 hours cercectomized
3 = 3 days cercectomized
8 = 8 days cercectomized
1 = 1 month cercectomized
5 = 5 months cercectomized
p >.1
*Measured on an 8000x Electron Micrograph.

Table 11. Ranking of Circumference of Adult Axons*

| Axon I | | Axon II | | Axon III | |
|---|---|---|---|---|---|
| 8 | 13.0 | C | 17.0 | 1 | 13.1 |
| C | 13.8 | 8 | 17.8 | 8 | 13.8 |
| 3 | 14.1 | 3 | 19.5 | C | 15.0 |
| 3 | 16.3 | 3 | 22.8 | 3 | 19.5 |
| 5 | 16.5 | 5 | 25.0 | 3 | 20.7 |
| 10 | 19.3 | 10 | 26.1 | 10 | 24.9 |
| 5 | 19.5 | 10 | 29.3 | 10 | 26.5 |
| 10 | 20.8 | 1 | 31.3 | 1 | 27.9 |
| 1 | 20.8 | 1 | 35.1 | C | 28.4 |
| 1 | 21.6 | 1 | 35.9 | 1 | 29.8 |
| C | 29.5 | C | 37.3 | 5 | 31.0 |
| C | 33.2 | C | 38.6 | 5 | 32.4 |
| 1 | 34.4 | C | 39.4 | C | 33.1 |
| C | 36.5 | | | C | 40.0 |

C = control
10 = 10 hours cercectomized
3 = 3 days cercectomized
8 = 8 days cercectomized
1 = 1 month cercectomized
5 = 5 months cercectomized
$p > .1$
*Measured in inches on an 8000x Electron Micrograph.

Table 12. Ranking of Circumference of Axons*

| NYMPHS | | | | | |
|---|---|---|---|---|---|
| Axon I | | Axon II | | Axon III | |
| 1 | 6.9 | 3 | 10.4 | 10 | 14.5 |
| 3 | 10.9 | 1 | 10.9 | C | 14.9 |
| 10 | 11.9 | 3 | 12.5 | 10 | 15.4 |
| 10 | 12.0 | 8 | 17.1 | 8 | 15.8 |
| C | 12.2 | 10 | 17.3 | 3 | 16.0 |
| 8 | 12.6 | C | 17.4 | 1 | 16.1 |
| 8 | 14.1 | 10 | 17.5 | 3 | 16.6 |
| 3 | 14.1 | 8 | 18.3 | 3 | 16.6 |
| 1 | 14.1 | 1 | 18.5 | 8 | 17.3 |
| 8 | 14.9 | 3 | 19.0 | 1 | 19.1 |
| 1 | 15.4 | 1 | 20.4 | 3 | 19.3 |
| 1 | 16.4 | 1 | 20.7 | 1 | 19.4 |
| 3 | 17.8 | 8 | 22.9 | 1 | 21.1 |
| 1 | 17.8 | 1 | 27.4 | C | 21.7 |
| | | | | 1 | 21.0 |
| | | | | 8 | 22.0 |

C = control
10 = 10 hours cercectomized
3 = 3 days cercectomized
8 = 8 days cercectomized
1 = 1 month cercectomized
5 = 5 months cercectomized
$p > .1$
*Measured in inches on an 8000x Electron Micrograph.

Table 13. Ranking of Younger and Older Nymph Cell and Nucleus Area Measurements*
(Paraffin Sections)

| YOUNGER | | | OLDER | | |
|---|---|---|---|---|---|
| Cell I | Cell II | Cell III | Nucleus I | Nucleus II | Nucleus III |
| 0 .9 | Y .8 | Y 1.0 | Y .1 | Y .2 | Y .2 |
| 0 .9 | 0 .8 | 0 1.0 | Y .2 | Y .2 | Y .3 |
| Y 1.0 | Y .9 | Y 1.2 | Y .2 | Y .2 | Y .3 |
| Y 1.0 | Y .9 | Y 1.2 | 0 .2 | Y .2 | 0 .3 |
| Y 1.0 | 0 .9 | 0 1.2 | 0 .2 | Y .2 | 0 .3 |
| 0 1.0 | Y 1.0 | Y 1.3 | 0 .2 | Y .2 | 0 .3 |
| 0 1.0 | Y 1.0 | Y 1.3 | 0 .2 | 0 .2 | 0 .3 |
| Y 1.1 | Y 1.0 | 0 1.3 | Y .3 | 0 .2 | 0 .3 |
| Y 1.1 | 0 1.0 | 0 1.4 | Y .3 | 0 .2 | Y .4 |
| 0 1.1 | 0 1.0 | 0 1.4 | Y .3 | 0 .2 | Y .4 |
| 0 1.1 | 0 1.0 | Y 1.5 | 0 .3 | 0 .2 | 0 .4 |
| 0 1.1 | Y 1.1 | Y 1.5 | 0 .3 | Y .3 | 0 .4 |
| Y 1.2 | 0 1.1 | 0 1.5 | 0 .3 | Y .3 | Y .5 |
| Y 1.2 | Y 1.2 | 0 1.5 | 0 .3 | 0 .3 | Y .5 |
| Y 1.2 | 0 1.2 | 0 1.7 | Y .4 | 0 .3 | 0 .5 |
| 0 1.2 | 0 1.2 | Y 1.8 | Y .4 | 0 .3 | 0 .5 |
| 0 1.2 | 0 1.3 | Y 1.8 | Y .4 | 0 .3 | 0 .5 |
| Y 1.3 | Y 1.4 | 0 1.9 | 0 .4 | Y .5 | 0 .5 |
| 0 1.3 | 0 1.9 | 0 1.9 | 0 .4 | 0 .5 | Y .6 |
| 0 1.4 | | | 0 .5 | 0 .5 | 0 .6 |

Y = Younger
0 = Older
$p > .1$
*Measured in $cm^2$ on an 400x drawing.

Table 14. Ranking of Number of Lysosomes in Nymph Cells*

| Cell I | Cell II | Cell III |
|---|---|---|
| C 4 | C 2 | C 1 |
| 1 4 | 1 6 | 1 3 |
| C 5 | C 7 | C 5 |
| 1 7 | 1 8 | 1 6 |
| 1 9 | 1 8 | 1 7 |
| C 10 | 1 9 | 1 8 |
| 1 10 | 1 10 | 1 8 |
| | C 11 | C 11 |

C = control
1 = 1 month cercectomized
$p > .1$
* Counted on an 8000x Electron Micrograph in Area 71.5$cm^2$.

Table 15. Ranking of Number of Lysosomes in Adult Cells*

| Cell I | Cell II | Cell III |
|---|---|---|
| C 4 | C 3 | 1 5 |
| 1 4 | 1 6 | C 6 |
| C 5 | 1 7 | 1 9 |
| 1 5 | 1 8 | 1 9 |
| C 7 | C 9 | C 10 |
| 1 8 | C 9 | C 11 |
| C 9 | 1 9 | C 13 |
| 1 9 | C 10 | 1 14 |
| C 13 | C 11 | |

C = control
1 = 1 month cercectomized
p >.1
* Counted on an 8000x Electron Micrograph in Area 71.5cm$^2$.

# CHAPTER V

## DISCUSSION AND CONCLUSIONS

The results reported herein indicate that the morphology of the somata and axons of three giant neurons in the last abdominal ganglion (A6) of both the nymph and adult cockroach was unaffected by massive removal of their sensory input. No qualitative or quantitative changes were seen. This is contrary to reported results of deafferentation of vertebrate neurons (Cowan, 1970; Globus, 1975). This apparent difference between the vertebrate and invertebrate nervous systems had led investigators to search for the explanation of these basic differences or to show that, in fact, there are no differences. The author and others (Murphey et al., 1975; Bentley, 1975) suspect that the problem arises from a lack of information rather than from the fact that the two types of neurons, vertebrate and invertebrate, are functionally different.

It has already been shown, primarily in vertebrates, that there is considerably more automony in a fully matured nervous system than in an immature, developing one (Jacobson, 1970). Young, developing vertebrate nervous systems react much more strongly to deafferentation than do those of adults.

So far, there are indications that this also may be true in invertebrate central nervous systems. One can compare the lack of reactive changes of deafferentation in adults (Tweedle et al., 1973,

Wine, 1973) with changes observed in juveniles (Bentley, 1975, Murphey et al., 1973; Murphey et al., 1975). I chose juvenile and adult animals to see if developing invertebrate neurons react more strongly than mature ones. On the one hand, the cockroach nymph interneurons might be considered mature in that their axons have made synaptic connections with their target cells. On the other hand, they might be considered immature and developing since they do not receive their total input until adulthood. With each molt, the instar develops additional sensory neurons in the cercus that project to the interneurons in the last abdominal ganglion (Edwards & Palka, 1973). Cowan (1970) summarized possible explanations for the greater susceptibility of immature neurons to deafferentation. According to Cowan (1970), "immature cells are in a metabolically unstable state and for this reason more susceptible to any form of noxious influence; as cells mature they become progressively more stable and hence more resistant to indirect injury." Alternatively, Cowan (1970) suggested that "immature cells may be more dependent upon some substance(s) derived from their afferents, and as the cells grow older they become increasingly self-sufficient and independent of such trophic influence" or that "immature cells which have not yet received all their afferents and whose axons have not yet established all their synaptic connections are unable to withstand the effects of denervation at this stage in their development."

The first possibility may be a plausible explanation for vertebrate systems in which it is common for axon terminal to make direct synaptic connections with the somata of their target cells.

The invertebrate interneurons of this study have no synaptic connections at the membrane of the somata. All synaptic interaction occurs in the neuropil. It may be that noxious degradation products of degenerating afferent axons and terminals simply do not reach the center of metabolic activity, the soma. However, if only the dendrites of these interneurons were affected, it would not have been observed in this study.

As to trophic influence, an increasingly persuasive body of evidence is accumulating for maintenance of muscle by nerve through diffusable "trophic substances" (Gutmann, 1976). Trophic substances also seem to play a role in nerve cell interactions within the nervous system (Harris, 1974). For example, Levi-Montalcini and Angeletti (1963) found that chick embryo spinal sensory and sympathetic ganglia neuroblasts do not grow in vitro in culture medium lacking nerve growth factor (NGF). That the hormone, NGF, has a trophic influence in neuronal development was shown by Levi-Montalcini and Booker (1960) who demonstrated that injection of an antiserum to NGF into newborn mice resulted in destruction of sympathetic nerve cells. It is possible for substances to move across synapses from one neuron to another. Upon injection of the radioactive amino acid, proline, into the eye of a mouse, Grafstein (1971) observed that the radioactive proline entered the retinal ganglion cells and was incorporated into protein. Radioactive assays of the lateral geniculate nucleus and visual cortex revealed the presence of a labeled protein. Apparently the protein, or some metabolic modification of it, had passed from the optic cup and along the axons of the optic nerve to the lateral

geniculate body. It was then transneuronally transferred to the visual cortex. Trumpy (1971) remarked that the neuronal degeneration observed in young animals in his study was similar to that seen by Levi-Montalcini utilizing antiserum to NGF. Furthermore, the nuclear changes observed with long-term exposure of neurons to antimetabolites which inhibit RNA synthesis were also similar. He suggested the possibility of transneuronal inhibition of RNA synthesis by deafferentation. If this is so, then perhaps some trophic substance in the form of an initiating factor for RNA or perhaps protein synthesis normally diffuses into the postsynaptic neuron and this diffusion stops when the presynaptic neuron is destroyed. This trophic substance may be significant in initiating RNA and protein synthesis in developing neurons and unimportant to mature neurons whose machinery for protein synthesis has already been "turned on." This explanation of trophic influences upon young versus mature neurons is not necessarily incongruous with the results of my results since some synaptic connections in nymphs had already been established before deafferentation. The neurons of the nymphs in my study could be regarded as "mature, in that their protein synthetic machinery is probably operating independently. Any postembryonic changes observed at this time then might only be at the level of the dendrites where changes have been observed in other studies (Bentley, et al., 1975; Murphey et al., 1975). This would also explain the lack of reactivity by adult neurons whose synaptic connections have all been established.

The third explanation advanced by Cowan appears to be an extension of the second; that is, trophic influences may be either

anterograde from presynaptic neurons or retrograde from postsynaptic neurons. If the explanation for invertebrate adult and juvenile differences lies in trophic interactions, then I am unable to explain why reactive changes, although less severe than in juveniles, occur in vertebrate adult neurons. It may be that various types of trophic interactions exist, rendering some cells more autonomous than others.

The theory has also been advanced that electrical activity is responsible for maintenance of postsynaptic neuronal integrity. It has been demonstrated in many vertebrates that functional deprivation leads to the type of transneuronal atrophy seen in surgical deafferentation but to a lesser extent (Globus, 1975). Adults are rarely affected by functional deprivation. This lack of reactivity in adult neurons tends to refute the theory of maintenance of postsynaptic neurons by electrical activity. Surgical deafferentation also has the effect of reducing the polarizing input. Perhaps ionic changes due to reduced synaptic excitation affect post-synaptic neuronal morphology. Cultured muscle cells can be maintained by artificial electrical stimulation in the absence of neuronal contact. Since the somata of the mutant cricket interneurons appeared "withered and markedly reduced in cell volume," Bentley, (1975), based on the fact that only the sensilla were absent while the sensory neurons were present concluded that electrical activity is necessary to maintain normal morphology. However, it may be possible that the mutation which affected the development of the sensilla may have also affected the sensory neuron since the sensory neuron and cells responsible for formation of the sensilla may come from division of a single

undifferentiated cell (Wigglesworth, 1953). If the sensory neurons were defective, the normal synaptic connections with the giant interneurons may not occur. The interneurons would be deprived of this synaptic input throughout embryonic development and not just post-embryonic development when some degree of autonomy is probably already achieved.

It has been suggested that completeness of deafferentation may be a complicating factor in the difficulties involved in making valid generalizations about the morphological features of transneuronal degeneration since the extent of degeneration seems to be directly related to the degree of deafferentation (Cowan, 1970). Wine (1973b) took exception to this. Six months after axotomy, although the crayfish medial giant axons had degenerated, the F-1 motor axons, contrary to expectation, showed no significant alterations in synaptic morphology at the level of light microscopy. Wine believed the medial giants may be the only source of excitatory synaptic input to the F-1 motor neurons. He did admit however, to the possibility of the existence of inhibitory input as demonstrated by Mittenthal and Wine (1973a) and that it could conceivably play a trophic function.

Although the cercal nerves provide the only known source of sensory input, the possibility also exists of other synaptic input to the three identified interneurons of this author's study. Milburn (1968) and Farley & Milburn (1969) have observed "satellite" nerve endings at each of the cercal-giant fiber synapses. These "satellite" fibers were presumed to be associated with the interneurons. Milburn and Bentley (1971) felt that these "satellite" fibers might possibly

be inhibitory intraganglionic interneurons. They cited the results of Callec & Boistel (1966) as supportive evidence for this since inhibitory potentials were often observed when the sensory neurons of the contralateral cercus were stimulated. Substrate vibration from an unidentified non-cercal source excites two of the largest interneurons in crickets (Edwards and Palka, 1974). If this were also true in cockroaches, the input from these substrate vibration receptors would not have been removed by cercectomy. Milburn & Bentley (1971), however, found these substrate vibration receptors to be located on the cerci. Since the possibility of other afferent input to the cockroach giant interneurons exists, the lack of reactive changes observed in this study may be due to maintenance by this source of input, small as it may be.

Finally, collateral sprouting of other nerves may have occurred replacing the degenerated cercal axon projections onto the interneuron dendrites. In vertebrates, intact nerves will send branches, or collateral sprouts, into degenerated or partially denervated nerve fields in both the peripheral and central nervous systems. Aguilar et al. (1973) mapped the peripheral fields of adult salamander spinal nerves 15, 16 &17. In a series of experiments, they severed nerve 16 on one side; applied colchicine to nerve 16 to retard fast axoplasmic flow; and severed nerve 16 in addition to applying colchicine to nerve 15. In the first case morphological, behavioral, and psysiological observations revealed collateral growth of nerves 15 & 17 into the fields normally occupied by nerve 16. Colchicine applied to nerve 16 resulted also in expansion of the fields of nerves 15 & 17.

When nerve 16 was cut and colchicine applied to nerve 15, only the field of nerve 17 seemed to expand. These results seemed to indicate that collateral sprouting did occur and that perhaps a trophic substance was involved. Aguilar et al. (1973) concluded that trophic substances, continually produced and released by neurons, help maintain normal dendritic fields by preventing invasion from neighboring nerves. When these trophic substances are no longer produced, neighboring nerves send out invading collateral sprouts. This was demonstrated by colchicine treatment of nerve 16 which was presumed to have stopped the flow of this trophic substance and therefore its release. Release apparently was stopped in nerve 15 with the application of colchicine since it did not invade the territory of severed nerve 16 as it had done before treatment with colchicine. Since collateral sprouting is known to occur, and no evidence to the contrary was seen in this study, it cannot be eliminated as a possible explanation for the lack of degenerative changes following deafferentation of interneurons I, II & III. It is unlikely, however, in view of the results of Murphey et al., (1975). Unilateral removal of a cercus still resulted in reactive changes of the deprived dendrites despite the possibility of collateral innervation as cercal fibers were observed to cross the other side of the ganglion where they normally do not occur.

Another result of this study was the lack of reaction of giant axons I, II and III to deafferentation. Considerable autonomy has been demonstrated in crayfish giant and motor axons severed from their somata (Hoy, 1967; Wine, 1973a). Cockroaches (Hess, 1958b) and

cricket (Edwards & Palka, 1971) giant axons degenerate very rapidly while severed cricket motor neuron dendrites maintain normal dendritic fields for about 60 days before degenerating (Clark, 1974). The dendritic field of cockroach motor neuron 28 showed a normal pattern 3 months after axotomy (Tweedle et al., 1973). Varied responses have been observed in locusts. Usherwood (1963) observed electrical activity in severed motor axons up to 84 days. Boulton and Rowell (1969) observed degeneration of axons in locust connectives within 2 days. The results of my study showed extensive degeneration of cercal nerve axons within 10 hours following cercectomy. There thus seems to be no inherent system of autonomy in the cockroach interneurons which could be used as an explanation for axons being unreactive to deafferentation. Since there is no direct synaptic input to the giant axons in the connective, they would be unaffected by degredation products of cercal axons and synaptic boutons. Further, since the somata appear to be unchanged and functioning normally, there would be no reason to expect the axons to behave differently. There may also be a trophic interaction between the giant fibers and the postsynaptic neurons upon which they form connections which would maintain morphological integrity of the giant interneurons. However, in view of the fact that degeneration of deafferented vertebrate neurons occurs and that axotomized cockroach axons degenerate, this last explanation is unlikely.

In summary, there was no morphological evidence of degeneration in cockroach giant interneurons I, II or III of the last abdominal ganglion following deafferentation. Several explanations for these

observations are possible. Firstly, if reactive changes did occur, but only at the level of the synapse, this would have gone undetected in the present study. Secondly, reactive changes did not occur because the adult and even nymph interneurons have achieved a mature, autonomous state. No electrical or trophic input would be necessary for maintenance. At this point, only direct physical injury to the neuron itself would produce reactive changes. Thirdly, there may be other synaptic input to the giant interneurons that was not removed upon cercectomy. This input may have been enough to maintain the morphological integrity of the interneurons. Finally, collateral sprouts may have grown into the synaptic areas vacated by the degenerating cercal axon terminals.

I have no direct evidence to refute any of the four alternatives just suggested so any one or perhaps a combination of two or more are possible explanations. All of these alternatives have been presented at some time to explain lack of expected reactions in vertebrate systems. It may be that when the complexity of the vertebrate nervous system is better understood, the apparent differences that now seem to exist between vertebrate and invertebrate nervous systems will be explained.

# CHAPTER VI

## PLATES

## Plate 1

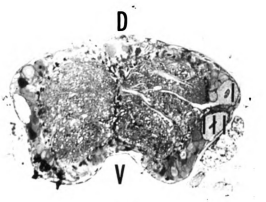
a. Last abdominal ganglion (A6). Nymph.
b. Last abdominal ganglion (A6). Adult.

D = dorsal
V = ventral

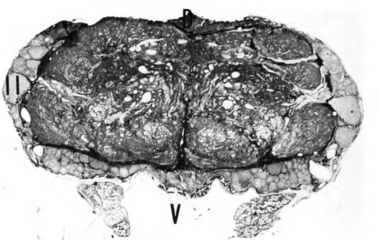
I = neuron I
II = neuron II
III = neuron III

Stained with methylene blue.

Photomicrographs. 115x.




a




b

Plate 1

## Plate 2

a. Connective between A5 and A6. Adult.
b. Last abdominal ganglion (A6). Nymph.

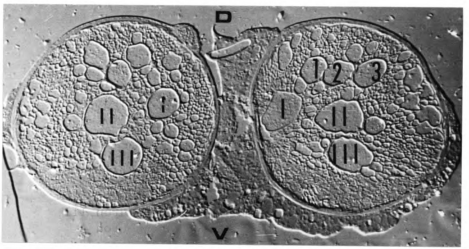
D = dorsal
V = ventral

| a. | | b. | |
|---|---|---|---|
| I | = axon I | I | = neuron I |
| II | = axon II | II | = neuron II |
| III | = axon III | III | = neuron III |
| 1 | = axon 1 | A | = neuron A |
| 2 | = axon 2 | B | = neuron B |
| 3 | = axon 3 | | |

'B' marks the location of neuron B which is out of the plane of focus.

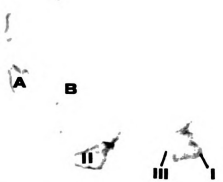
a. Stained with methylene blue. Nomarski differential interface optics.
b. Cells stained by diffusion of cobalt into the axons.

Photomicrograph a. 288x.
Photomicrograph b. 115x.




a

II




b

Plate 2

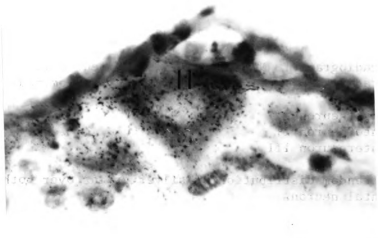
## Plate 3

a. Autoradiography. Normal adult. Interneuron II.
b. Autoradiography. Adult. Three day cercectomized. Interneuron I & II.

I = Interneuron I
II = Interneuron II
III = Interneuron III

Note the random distribution of silver grains over both the control and experimental neurons.

Photomicrograph. 720x.



a

b

Plate 3

## Plate 4

Adult. Normal. Interneuron I.

Gl = glial sheath
Go = Golgi complex
L = lysosome
Li = "lipochondria"
M = mitochondria
NS = Nissl substance
T = trophospongium

Electron micrograph. 8000x.

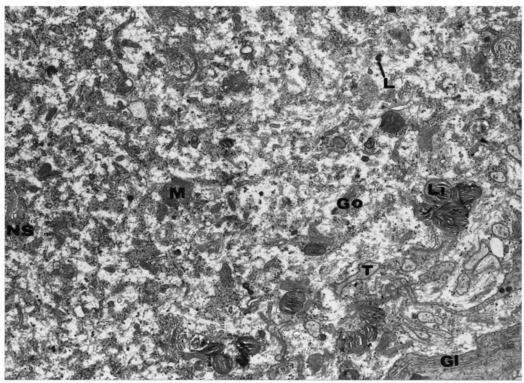



Plate 4

Plate 5

Adult. Normal. Interneuron II.

G = glycogen
Gl = glial sheath
L = lysosome
Li = "lipochondrion"
M = mitochondria
N = nucleus
NS = Nissl substance
T = trophospongium

Note that the glycogen deposit is on the periphery of the cell.

Electron micrograph. 8000x.

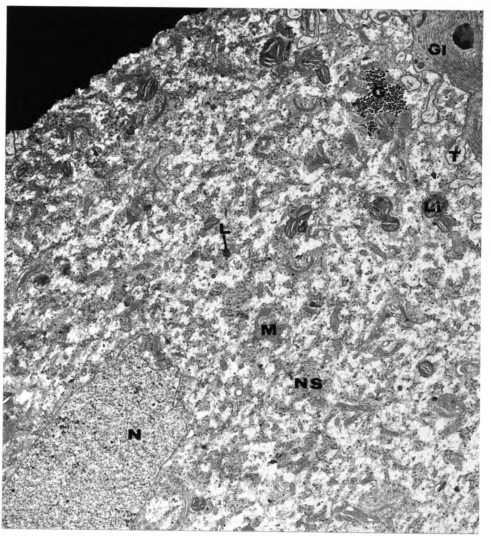



Plate 5

## Plate 6

Adult. Normal. Interneuron III.

G = glycogen
Gl = glial sheath
Go = Golgi complex
Li = "lipochondrion"
MV = multivesicular body
NS = Nissl substance
T = trophospongium

Note the partially depleted glycogen deposit with associated mitochondria and "lipochondria" in about equal abundance.

Electron micrograph. 8000x.

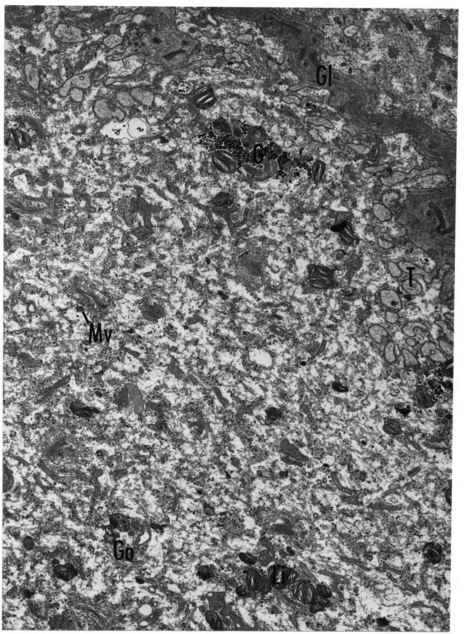



Plate 6

## Plate 7

Adult. One month cercectomized. Interneuron I.

Gl = glial sheath
Go = Golgi complex
L = lysosome
Li = "lipochondrion"
M = mitochondria
N = nucleus
NS = Nissl substance
T = trophospongium
RER= rough endoplasmic reticulum

Note the close association of mitochondria with the Nissl substance.

Electron micrograph. 8000x.

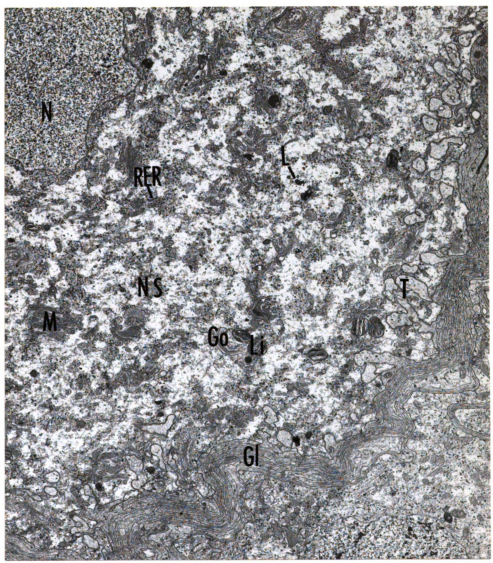



Plate 7

## Plate 8

Adult. One month cercectomized. Interneuron II.

G = glycogen
Lm = "lipochondrion" containing mitochondrial fragments
M = mitochondria
R = free ribosomes
SER= smooth endoplasmic reticulum

Note that almost totally depleted glycogen deposit infiltrated with large "lipochondria." Also apparent are "lipochondria" containing fragments of mitochondria (Lm).

Electron micrograph. 13,000x.

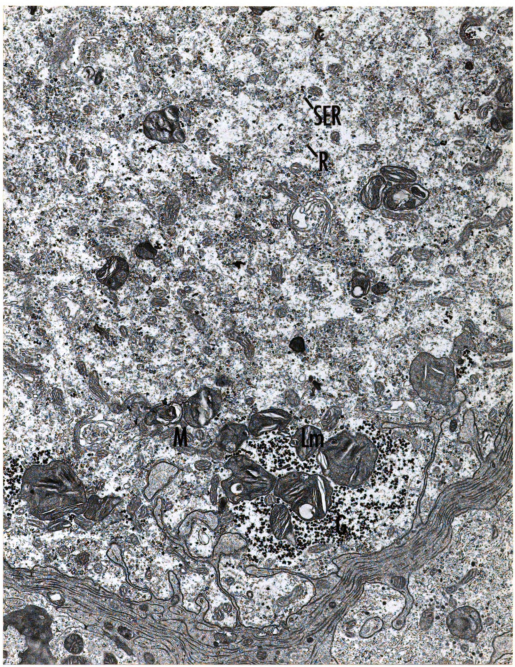



Plate 8

## Plate 9

Nymph. Normal. Interneuron I.

Gl = glial sheath
Go = Golgi complex
L = lysosome
Li = "lipochondrion"
M = mitochondrion
N = nucleus
Ni = lightly staining fibrous nuclear inclusion
NS = Nissl substance
T = trophospongium

Note the lightly staining nuclear inclusion (Ni). Compare the invasion of the trophospongium with that of the one month cercectomized nymph interneuron I (Plate 12).

Electron micrograph. 8000x.

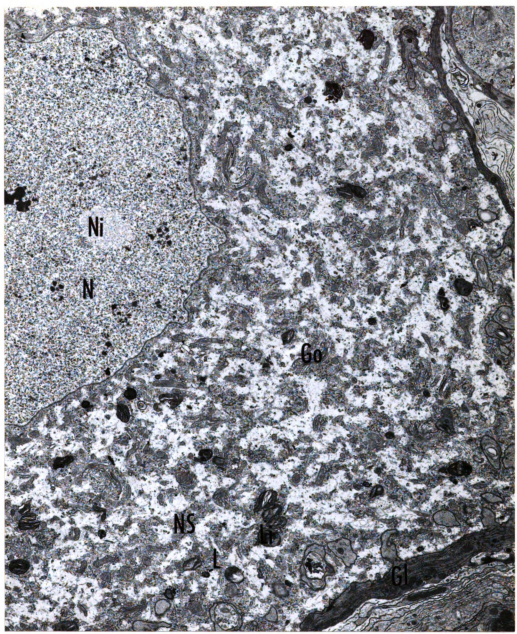



Plate 9

Plate 10

Nymph. Normal. Interneuron II.

Gl = glial sheath
Go = Golgi complex
L = lysosome
Li = "lipochondrion"
M = mitochondria
NS = Nissl substance
T = trophospongium

Note the dilations of several of the cisternae at the convex face of the Golgi complex. Also note the close association of mitochondria with Golgi bodies and Nissl substance.

Electron micrograph. 8000x.

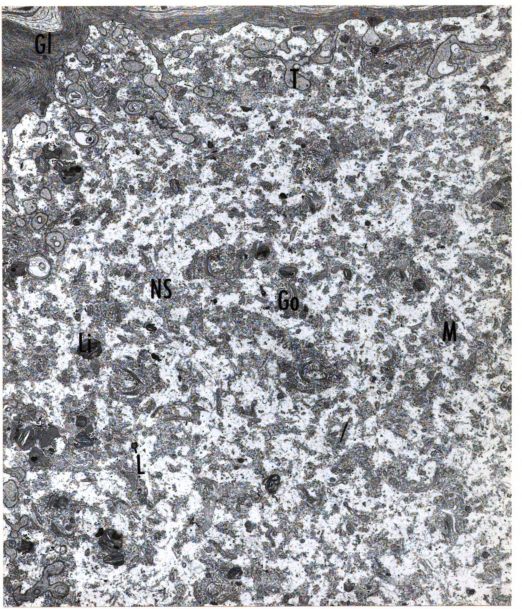



Plate 10

## Plate 11

Nymph. Normal. Interneuron III.

Gl = glial sheath
Go = Golgi complex
L = lysosome
Li = "lipochondrion"
M = mitochondria
N = nucleus
NS = Nissl substance
T = trophospongium

Electron micrograph. 8000x.

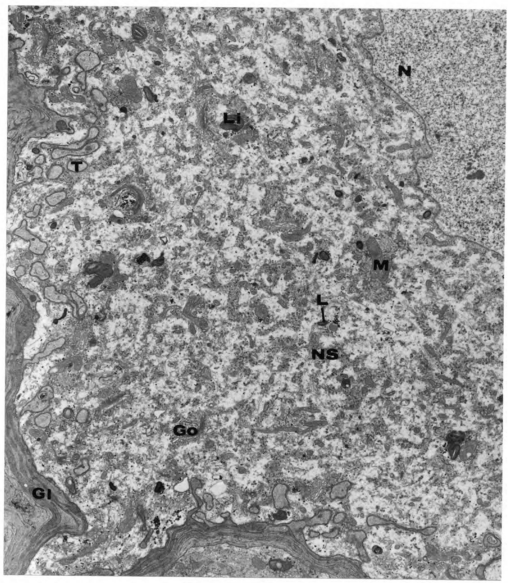



Plate 11

## Plate 12

Nymph. One month cercectomized. Interneuron I.

Gl = glial sheath
Go = Golgi complex
L = lysosome
Li = "lipochondrion"
M = mitochondria
MV = multivesicular body
NS = Nissl substance
T = trophospongium

Note the aggregates of Nissl substance closely associated with the nucleus and also scattered throughout the cytoplasmic ground substance. Mitochondria are also frequently found in abundance near the nucleus.

Electron micrograph. 8000x.

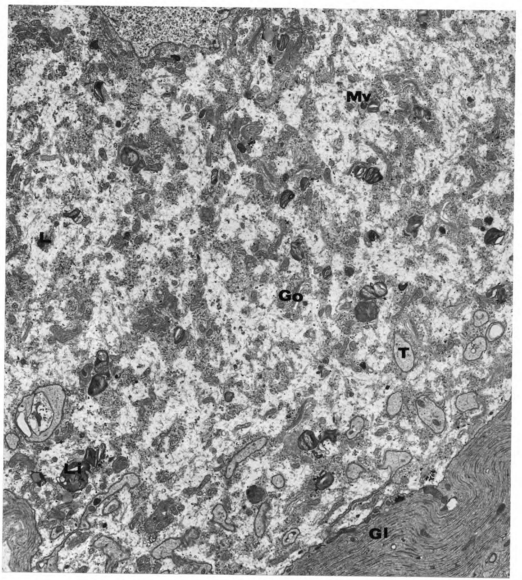



Plate 12

## Plate 13

Nymph. One month cercectomized. Interneuron III.

Gl = glial sheath
Go = Golgi complex
L = lysosome
Li = "lipochondrion"
M = mitochondria
NS = Nissl substance
T = trophospongium

Note that more Golgi bodies are seen toward the periphery and that they seem to exhibit no preferred orientation. Also note that close association of lysosomes with the concave face of the Golgi complex.

Electron micrograph. 8000x.

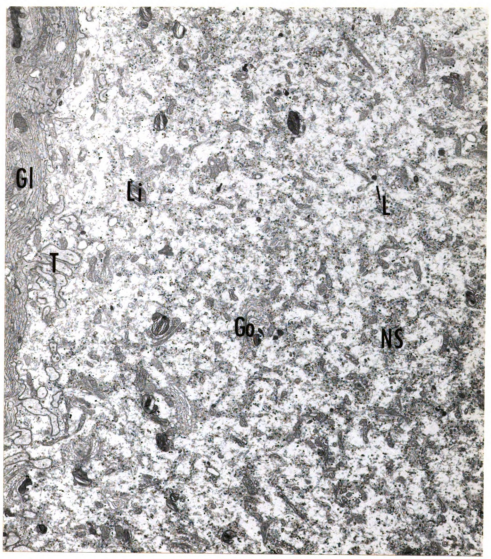



Plate 13

## Plate 14

Adult. Normal. Axon III.

Gl = glial sheath
M = mitochondrion

Note that most of the mitochondria are seen in cross section. Compare the thickness of the glial sheath this axon with that of axon III of an eight day cercectomized animal (Plate 15).

Electron micrograph. 8000x.

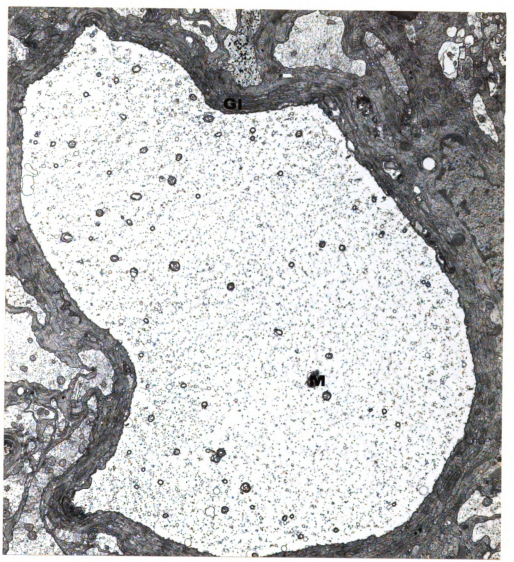



Plate 14

## Plate 15

Adult. Eight day cercectomized. Axon III.

Gl = glial sheath
In = glial invaginations
M = mitochondrion

Note the thickness of the glial sheath is comparable to that of the normal animal (Plate 14). Several small glial sheath invaginations can be seen extending into the axoplasm (In).

Electron micrograph. 8000x.

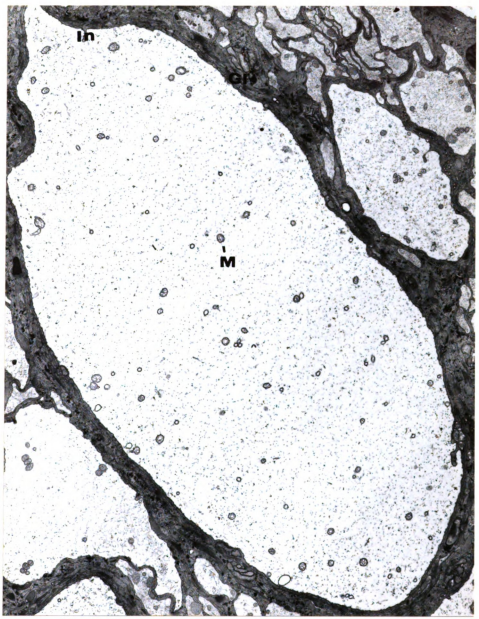



Plate 15

## Plate 16

Nymph. Normal. Axon I.

Gl = glial sheath
M = mitochondrion

Note the random and homogeneous dispersion of the axoplasmic elements: mitochondria, microtubules, and microfilaments. Compare the thickness of the glial sheath of this axon with that of axon I of a one month cercectomized animal (Plate 17).

Electron micrograph. 8000x.

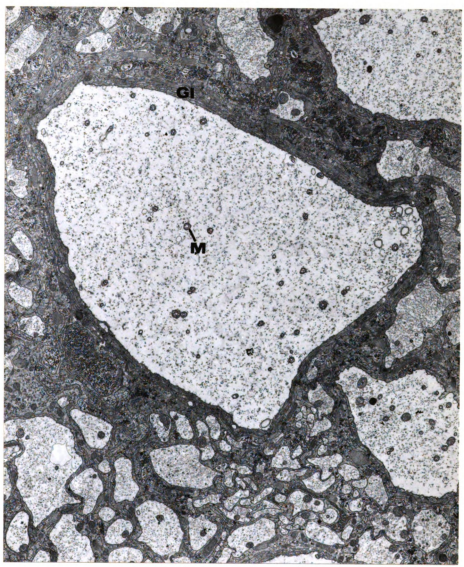



Plate 16

Plate 17

Nymph. One month cercectomized. Axons I and III.

I = Axon I
III = Axon III
Gl = glial sheath
M = mitochondrion

Note that most of the mitochondria are viewed in cross section.

Electron micrograph. 8000x.

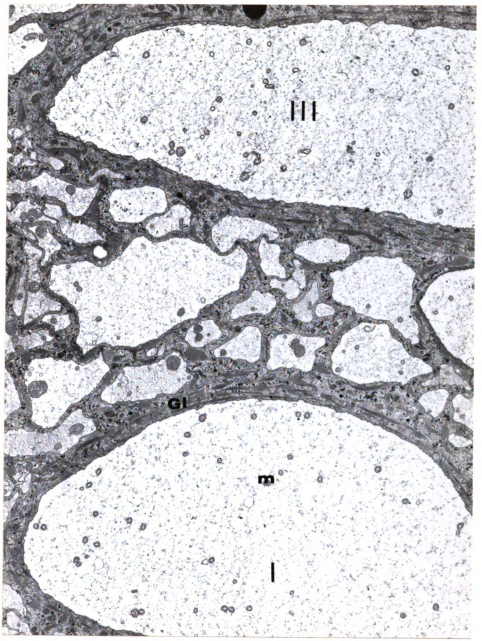



Plate 17

Plate 18

Nymph. Three day cercectomized. Neuropil.

Co = neurite containing cobalt - DAB complex
D = degenerating neurite
No = normal neurite

Compare the distorted ultrastructure of neurites containing the cobalt - 3,3' -diaminobenzidine (DAB) complex (Co) to normal neurites (No). This micrograph is from a three day cercectomized animal so degenerating cercal fibers (D) are seen. The distortion by cobalt-DAB, however, is not a degenerative reaction to cercectomy as the same distortion is also seen in normal animals.

Electron micrograph. 37,500x.

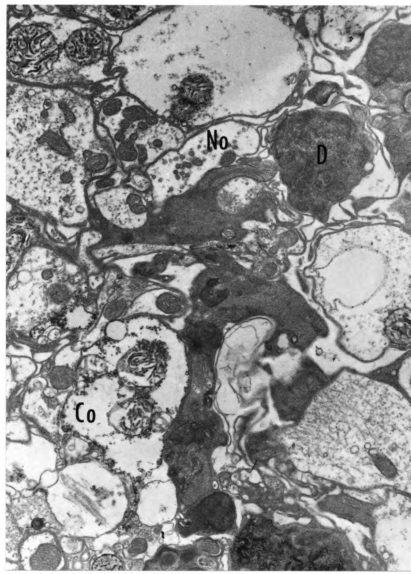



Plate 18

# REFERENCES

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Aguilar, C.E., M.A. Bisby, E. Cooper, and J. Diamond, 1973. "Evidence that axoplasmic transport of trophic factors is involved in the regulation of peripheral nerve fields in salamanders." J. Physiol. 234:449-464.

Aleu, F.P., R.D. Terry, & H. Zellweger, 1965. "Electron microscopy of two cerebral biopsies in gargoylism." J. Neuropathol. Exp. Neurol. 24:304-317.

Baker, J.R., 1963. "New developments in the Golgi controversey." J. Roy. Microscop. Soc. 82:145-157.

Bentley, D., 1975. "Single gene cricket and mutations: effects on behavior, sensilla, sensory neurons, and identified interneurons." Science 187:760-764.

Boulton, P.S. & C.H.F. Rowell, 1969. "Degeneration and regeneration in the insect central nervous system." II Z. Zellforsch 101:119-134.

Bullock, T.H. & G.A. Horridge, 1965. Structure and Function in the Nervous System of Invertebrates Vol. I. W.H. Freeman & Co., San Francisco, 798 pp.

Byers, M.R., 1970. "Chromatolysis in a pair of identifiable metathoracic neurons in the cockroach, Diploptera punctata." Tissue & Cell 2:255-279.

Callec, J.-J. & J. Boistel, 1965. "Analysis with microelectrodes of the synaptic transmission at the level of the sixth abdominal ganglion of a cockroach, Periplaneta americana." In: J.E. Treherne & J.W.L. Beament (Eds.), The Physiology of the Insect Central Nervous System Academic Press, N.Y., pp. 59-65.

Callec, J.-J. & J. Boistel, 1966. "Phénomènes d'excitation et d'inhibition au niveau du dernier ganglion abdominal de la Blatte, Periplaneta americana L.

Chou, J.T.Y. & G.A. Meek, 1958. "The ultra-fine structure of lipid globules in the neurons of Helix aspersa." Quart. J. Microscop. Sci. 99:279-284.

Clark, R.D., 1974. "Dendritic stability without soma in an identified arthropod motor neuron." Amer. Zool. 14:1272.

Cohen, M.J., 1967. "Correlations between structure, function and RNA metabolism in central neurons of insects." In C.A.G. Wiersma (ed.), Invertebrate Nervous Systems. University of Chicago Press, Chicago. pp. 65-78.

Cohen, M.J., 1970. "A comparison of invertebrate and vertebrate central neurons." In: F.O. Schmitt (ed.), The Neurosciences: Second Program. Rockefeller University Press, N.Y. pp. 798-811.

Cohen, M.J. & J.W. Jacklet, 1965. "Neurons of insects: RNA changes during injury and regeneration." Science 148:1237-1239.

Cohen, M.J. & J.W. Jacklet, 1967. "The functional organization of motor neurons in an insect ganglion." Phil. Trans. Roy. Soc. Lond. B 252:561-569.

Cowan, W.M., 1970. "Anterograde and retrograde transneural degeneration in the central and peripheral nervous system." In: W.J.H. Nauta and S.O.E. Ebbesson (eds.), Contemporary Research Methods in Neuroanatomy. Springer-Verlag, N.Y. pp. 217-251.

Cragg, B.G., 1970. "What is the signal for chromatolysis?" Brain Res. 23:1-21.

Dagan, D. & K. Parnas, 1970. "Giant fiber and small nerve pathways involved in the evasive response of the cockroach, Periplaneta americana." J. Exp. Biol. 52:313-324.

Edwards, J.S. and J. Palka, 1971. "Neural regeneration delayed formation of central contacts by insect sensory cells." Science 172:591-594.

Edwards, J.S. & J. Palka, 1973. "Neural specificity as a game of cricket: some rules for sensory regeneration in Acheta domesticus." In: D. Young and (ed.), Developmental Neurobiology of Arthropods. Cambridge University Press, London. pp. 131-146.

Edwards, J.S. and J. Palka, 1974. "The cerci and abdominal giant fibers of the house cricket, Acheta domesticus." Proc. Roy. Soc. Lond. B. 185:83-103.

Farley, R.D. & N.S. Milburn, 1969. "Structure and function of the giant fiber system in the cockroach, Periplaneta americana." J. Insect Physiol. 15:457-476.

Ghetti, B., D.S. Horoupian, & H.M. Wísniewski, 1972. "Trans-synaptic response of the lateral geniculate nuclues and the pattern of degeneration of the nerve terminals in the rhesus monkey after eye enucleation." Brain Res. 45:31-48.

Gillette, R. & B. Pomeranz, 1973. "Neuron geometry and circuitry via the electron microscope: intracellular staining with osmiophilic polymer." Science 182:1256-1257.

Globus, Albert, 1975. "Brain morphology as a function of pre-synaptic morphology and activity." In: A.H. Riesen (ed.), The Developmental Neuropsychology of Sensory Deprivation. Academic Press, N.Y., pp. 9-91.

Grafstein, B., 1971. "Transneuronal transfer of radioactivity in the central nervous system." Science 172:177-179.

Guthrie, D.M. & A.R. Tindall, 1968. The Biology of the Cockroach. St. Martin's Press, N.Y., 408 pp.

Gutman, E., 1976. "Neurotrophic relations." Ann. Rev. Physiol. 38:177-216.

Harris, A.J., 1974. "Inductive functions of the nervous system." Ann. Rev. Physiol. 36:251-303.

Harris, C.L. & T. Smyth, Jr., 1971. "Structural details of cock-roach giant axons revealed by injected dye." Comp. Biochem. Physiol. 40A:295-303.

Hess, A., 1958a. "The fine structure of nerve cells and fibers, neuroglia, and sheaths of the ganglion chain in the cockroach (Periplaneta americana)." J. Biophys. Biochem. Cytol. 4:731-742

Hess, A., 1958b. "Experimental anatomical studies of pathways in the bers, severed central nerve cord of the cockroach." J. Morph. 103:479-502.

Hess, A., 1960. "The fine structure of degenerating nerve fibers, their sheaths, and their terminations in the central nerve cord of the cockroach (Periplaneta americana)." J. Biophys. Cytol. 7:339-344.

Horridge, G.A., 1973. "Neuron constancy and connection patterns in functional and growth studies." In: D. Young (ed.), Developmental Neurobiology of Arthropods. Cambridge University Press, London pp. 233-257.

Hoy, R., 1967. "Degeneration and regeneration in the crayfish abdominal flexor system." J. Gen. Physiol. 50:2482.

Humason, G.L., 1967. Animal Tissue Techniques. W.H. Freeman & Co., San Francisco. 569 pp.

Jacklet, J.W. & M.J. Cohen, 1967. "Nerve regeneration: correlation of electrical, histological, and behavioral events. Science. 156:1640-1643.

Jacobson, J., 1970. Developmental Neurobiology. Holt, Rinehart, & Winston, Inc., N.Y. 465 pp.

Jean-Baptiste, M. & D.K. Morest, 1975. "Transneuronal changes of synaptic endings and nuclear chromatin in the trapezoid body following cocklear ablations in cats." J. Comp. Neurol. 162:111-134.

Jones, W.H. & D.B. Thomas, 1962. "Changes in the dendritic organization of neurons in the cerebral cortex following deafferentation." J. Anat. (Lond.) 96:375-381.

Karnovsky, M.J., 1965. "A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy." J. Cell Biol. 27:137A.

Kennedy, D., A.I. Selverston, & M.P. Remler, 1969. "Analysis of restricted neural networks." Science 164:1488-1469.

Kerkut, G.A., R.M. Pitman, & R.J. Walker, 1969. "Iontophoretic application of acetylocholine and GABA onto insect central neurons." Comp. Biochem. Physiol. 31:611-633.

Kurnick, N.B., 1955. "Pyronin Y in the methyl green-pyronin histological stain." Stain Technol. 30:213-230.

Lane, N.J., 1968. "Lipochondria, neutral red granules and lysosomes: synonymous terms?" In: S.M. McGee-Russell & K.F.A. Ross (eds.), Cell Structure and its Interpretation. St. Martins Press, N.Y., pp. 169-182.

Levi-Montalcini, R. & B. Booker, 1960. "Destruction of the sympathetic ganglia in mammals by an antiserum to the nerve-growth promoting factor." Proc. Nat. Acad. Sci. U.S. 42:384-391.

Levi-Montalcini, R. & P.U. Angeletti, 1963. "Essential role of the nerve growth factor in the survival and maintenance of dissociated sensory and sympathetic nerve cells in vitro." Develop. Biol. 7:653-659.

Loewy, A.D., 1970. "A study of neuronal types in Clarke's column in the adult cat." J. Comp. Neurol. 139:53-80.

Loewy, A.D., 1972. "The effects of dorsal root lesions on Clark neurons in cats of different ages." J. Comp. Neurol. 145:141-164.

Matthews, M.B. and T.P.S. Powell, 1962. "Some observations on transneuronal cell degeneration in the olfactory bulb to the rabbit." J. Anat. (Lond.), 96:89-101.

Mendenhall, B. & R.K. Murphey, 1974. "The morphology of cricket giant interneurons." J. Neurobiol. 5:565-580.

Milburn, N.S., 1968. "Fine structure of the cercal nerve-giant fiber synapse in the sixth abdominal ganglion of Periplaneta americana." Amer. Zool. 8:778.

Milburn, N.S. & D.R. Bentley, 1971. "On the dendritic topology and activation of cockroach giant interneurons." J. Insect Physiol. 17:607-623.

Mittenthal, J.E. & J.J. Wine, 1973. "Connectivity patterns of crayfish giant interneurons: visualization of synaptic regions with cobalt dye." Science 179:182-184.

Murphey, R.K., 1973. "Characterization of an insect neuron which cannot be visualized in situ." In: Kater, S.B. & C. Nicholson (eds.), Intracellular Staining in Neurobiology. Springer-Verlag, N.Y., pp. 135-150.

Murphey, R.K., B. Mendenhall, J. Palka, and J.S. Edwards, 1975. "Deafferentation slows the growth of specific dendrites of identified giant interneurons." J. Comp. Neurol. 159:407-418.

Murphey, R.K., J. Palka, & J.S. Edwards, 1973. "Effects of sensory deprivation on the geometry of an identified giant interneuron in crickets." Amer. Zool. 13:1302.

Palka, J. & J.S. Edwards, 1974. "The cerci and abdominal giant fibers of the house cricket, Acheta domesticus. II. Regeneration and effects of chronic deprivation." Proc. Roy. Soc. Lond. B. 185:105-121.

Parnas, I. & D. Dagan, 1971. "Functional organization of giant axons in the central nervous system of insects: new aspects." Adv. Insect Physiol. 8:95-144.

Pinching, A.J. and T.P.S. Powell, 1971. "Ultrastructural features of transneuronal cell degeneration in the olfactory system." J. Cell Sci. 8:253-287.

Pitman, R.M., C.D. Tweedle, and M.J. Cohen, 1972a. "Branching of central neurons: intracellular cobalt injection for light and electron microscopy." Science 176:412-414.

Pumphrey, R.J. & A.F. Rawdon-Smith, 1937. "Synaptic transmission of nervous impulses through the last abdominal ganglion of the cockroach." Proc. Roy. Soc. Lond. B. 122:106-118.

Rasch, E., H. Swift, A.H. Riesen, & K.L. Chow, 1961. Altered structure and composition of retinal cells in dark-reared mammals.

Roeder, K.D., 1948. "Organization of the ascending giant fiber system in the cockroach (Periplaneta americana). J. Exp. Zool. 108:243-261.

Rustioni, A. and C. Sotelo, 1974. "Some effects of chronic deafferentation on the ultrastructure of the nucleus gracilis of the cat." Brain Res. 73:527-533.

Smith and Treherne, 1963. "Functional aspects of the organization of the insect nervous system." Adv. Insect Physiol. 1:401-484.

Spurr, A.R., 1969. "A low-viscosity epoxy resin embedding medium for electron microscopy." J. Ultrastructure Res.

Stretton, A.O.W. & E.A. Kravitz, 1968. "Neuronal geometry: determination with a technique of intracellular dye injection." Science 162:132-134.

Trumpy, J.H., 1971. "Transneuronal degeneration in the pontine nuclei of the cat." Ergebn. Anat. Entwick. 44:1-72.

Tweedle, C.D., R.M. Pitman, & M.J. Cohen, 1973. "Dendritic stability of insect central neurons subjected to axotomy and deafferentation." Brain Res. 60:471-476.

Usherwood, P.N.R., 1963. "Response of insect muscle to degeneration. I. Resting potential changes." J. Insect Physiol. 9:247-255.

Venable, J.H. and R. Coggeshall, 1965. "A simplified lead citrate stain for use in electron microscopy." J. Cell Biol. 25:407-408.

Wigglesworth, V.B., 1953. "The origin of sensory neurons in an insect, Rhodnius prolixus (Hemiptera)." Quart. J. Microscop. Sci. 94:93-112.

Willey, R.B. & G.B. Chapman, 1962. "Fine structure of neurons within the pars intercerebralis of the cockroach, Blaberus craniifer." Gen. Comp. Endocrinol. 2:31-43.

Wine, J.J., 1973a. "Invertebrate central neurons: orthograde degeneration and retrograde changes after axotomy." Exp. Neurol. 38:157-169.

Wine, J.J., 1973b. "Invertebrate synapse: long term maintenance of postsynaptic morphology following denervation." Exp. Neurol. 41:649-660.

Young, D., D.E. Ashurst, & M.J. Cohen, 1970. "The injury response of the neurons of Periplaneta americana." Tissue & Cell 2:387-398.