METABOLIC CHANGES IN DEAFFERENTED CENTRAL NEURONS OF AN INSECT, *ACHETA DOMESTICUS*

I. Effects upon Amino Acid Uptake and Incorporation¹

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Abstract

Chronic cercal deafferentation of the terminal ganglion in developing crickets (*Acheta domesticus*), which is known to suppress normal development of giant interneuron dendritic arborizations, is shown here to reduce [³H]leucine uptake and incorporation into ganglion proteins. Short term deafferentation of adult crickets, in contrast, does not depress amino acid uptake and incorporation significantly.

Following unilateral long term deafferentation of the terminal ganglion, a comparison was made of the [³H]leucine incorporation into primary dendritic processes and somata of deafferented and normally innervated medial giant interneurons (MGIs) within the same ganglion by means of quantitative autoradiography. Grain densities within dendrites of deafferented MGIs were significantly lower than in paired control MGIs; grain densities within somata of deafferented MGIs also were reduced, although the effects of deafferentation were less pronounced in somata than in target dendrites. These results imply a specific influence of afferent innervation on protein metabolism during growth and development of target postsynaptic elements.

The state of afferent innervation can influence the growth and development of some target neurons in the central nervous system profoundly (Cowan, 1970; Smith, 1977). Deafferentation at various, but usually at relatively early, stages of neural development can result in partial or complete atrophy of postsynaptic target cells (transneuronal degeneration) (Globus and Scheibel, 1967; Kelly and Cowan, 1972; Lynch et al., 1974). In contrast, some neurons remain remarkably resistant to deafferentation and display little or no apparent reaction to the loss of presynaptic connections (Purves, 1976). Certain invertebrate neurons have been shown to undergo varying degrees of degeneration following deafferentation (Kutsch, 1974; Bentley, 1975; Macagno, 1979), yet extreme stability to denervation has come to be regarded as a feature of many invertebrate central neurons (Tweedle et al., 1973; Clark, 1977; Krasne and Lee, 1977; Meyer

and Bittner, 1978a). Although it has been difficult to predict *a priori* how given target neurons will respond to denervation, it seems probable that those which undergo retardation of growth or degenerative changes are highly dependent upon the presence of intact synapses or corresponding trans-synaptic influences.

The cercal sensory-giant interneuron pathway in the terminal abdominal ganglion of the house cricket *Acheta domesticus* (Fig. 1) has been described in detail with respect to morphology, physiology, and development (Edwards and Palka, 1974; Palka and Edwards, 1974). Identifiable giant interneurons in the terminal abdominal ganglion receive the majority of their known sensory input from cercal sensory axons with cell bodies located in the caudal cercal appendages (abdominal cerci). During postembryonic development and cercal growth, increasing numbers of sensory axons project to, and synapse with, specific central neurons. These events continue throughout subsequent postembryonic stages.

The growth of identifiable cricket giant interneurons, in contrast to some invertebrate central neurons, is retarded by removal of cercal sensory axons during postembryonic development (Murphey et al., 1975; Murphey and Levine, 1980). Chronic deafferentation produces detectable physiological and morphological changes in target interneurons in the terminal ganglion, including no-

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ticeably decreased growth of primary and secondary dendritic processes and dendrite spines. These effects were shown to be more dependent upon the actual presence rather than the activity of afferent input (Murphey, 1977). Although neural development is undoubtedly regulated by a variety of intracellular mechanisms, the question arises as to whether transneuronal metabolic interactions also may be involved in the growth of cricket interneurons and may account for the effects of deafferentation observed in the cricket CNS.

In this study, we investigated the possible effects of deafferentation on the metabolism of specific target giant interneurons in the cricket CNS. The results indicate that amino acid uptake and incorporation into ganglion proteins are altered markedly by chronic, but not by short term, deafferentation. More specifically, [^3H]leucine incorporation within somata and dendrites of identifiable target interneurons is reduced following denervation. Our findings suggest that transneuronal interactions may play a role in the development and maintenance of central neurons through effects upon their metabolism.

Materials and Methods

Deafferentation. Surgical removal of cerci at various stages of development effectively eliminates the majority of afferent input onto the dendrites of target giant interneurons. Degeneration of sensory axons and synaptic terminals occurs rapidly; pronounced degeneration of sensory axon terminals is seen as early as 4 to 6 hr after cercectomy and may be nearly complete by 12 hr (J. S. Edwards, unpublished observations).

Procedures for rearing, maintaining, and surgically treating immature and adult crickets have been presented elsewhere (Palka and Edwards, 1974) and generally were followed for the current studies. The animals used were obtained from the same clutches and care was taken to rear control and cercectomized groups under identical conditions (including sham operations on control groups where appropriate) to minimize asynchronous growth between groups. Adult body weight and size were not significantly different between control and operated groups when the absence of cerci was considered.

Three deafferentation paradigms were used. The most extreme form of deafferentation was continuous bilateral removal of cerci from hatching through all immature developmental instars to adulthood. These animals were designated the *long term symmetrical absent* (S.A._L) group. Regenerate buds were removed surgically from anesthetized animals following each molt. In another group, normal postembryonic development was permitted to occur and surgical deafferentation was deferred until adult maturity was reached (i.e., *short term symmetrical absent* (S.A._s) group). Normally reared (control) animals were termed the *symmetrical continuous* (S.C.) group. For the remaining experiments, groups of animals were raised in which only one cercus was extirpated throughout development; the remaining contralateral cercus was allowed to develop normally. This was the *asymmetrical continuous* (A.C.) group which was utilized for autoradiographic experiments allowing the comparison of normal and deafferented medial giant interneurons within the same ganglion.

Amino acid incorporation. Terminal ganglia were removed surgically from animals while they were cooled to $\sim 4^\circ\text{C}$ on ice. Ganglia were transferred to a solution of HEPES-buffered Levine saline solution, pH 7.4 (HBL; Levine, 1966), into which 95% O_2 , 5% CO_2 was bubbled continuously. Ganglia were preincubated at room temperature for 30 min prior to transfer to a fresh solution of HBL containing 50 or 100 $\mu\text{Ci ml}^{-1}$ of [^3H]l-leucine (60 Ci mmol^{-1}). In most experiments, an incubation time of 1 hr was chosen to allow sufficient uptake and incorporation for detection by liquid scintillation spectrometry and autoradiography. In some experiments, incorporation was inhibited by the addition of either cycloheximide (200 $\mu\text{g ml}^{-1}$) or puromycin dihydrochloride (100 $\mu\text{g ml}^{-1}$) to the preincubation and incubation media.

Trichloroacetic (TCA) acid-soluble and -insoluble counts were assessed per ganglion by filter paper-binding microassay (Mans and Novelli, 1961). Individual ganglia were removed rapidly from the incubation medium, rinsed in ice cold HBL, transferred to test tubes, and homogenized with a ground glass pestle. Homogenates were resuspended in 400 μl of HBL and a 100- μl aliquot was withdrawn for protein determination using a dye-binding microassay (Bradford, 1976). The remaining aliquots of homogenate were dispensed onto Whatman GF/C filter disks and air-dried. Disks were placed into Aquasol (with 1% Triton X-100) and counted in a liquid scintillation spectrometer (30% efficiency) either directly after drying to determine total radioactivity or after two 1-hr extractions, each with hot (90°C) and ice cold 10% TCA, followed by 1-hr extractions in chloroform/methanol and ether to determine incorporation as expressed as TCA-insoluble specific activity. [^3H]Leucine uptake, expressed as TCA-soluble activity, was determined by subtracting TCA-insoluble activity from the total radioactivity per ganglion.

Target interneuron localization and autoradiography. Ganglia from A.C. and S.C. animals were incubated in [^3H]leucine as described, rinsed in fresh HBL, and fixed for 2 hr in a solution of 0.5% glutaraldehyde, 2% paraformaldehyde in Millonig's buffer, pH 7.4. Dehydration in an ascending ethanol series was followed by embedment in JB-4 glycol methacrylate resin (Polysciences) and 1- μm horizontal serial sections were placed on slides. Slides were coated with Kodak NTB-2 liquid emulsion (1:1 in water), exposed at 4°C in sealed containers for 18 hr to 1 week, developed in Kodak Dektol (1:2; 16°C) for 3 min, and fixed. Slides were air-dried and stained with Richardson's stain.

The medial giant interneuron (MGI; interneuron 8-1), a known postsynaptic target of cercal sensory projections (Matsumoto and Murphey, 1977), was selected for autoradiographic analysis of intracellular labeling due to its distinctive morphology and topographic location in the terminal ganglion. MGI somata and primary dendritic processes contralateral to the somata were identified in serial sections of terminal ganglia based upon data from previous anatomical studies of cobalt-stained MGI (Mendenhall and Murphey, 1974; Murphey et al., 1975).

MGI somata were readily identifiable by their large ($\geq 40 \mu\text{m}$) diameter, distinctive cytoplasmic granularity, and extreme dorsolateral placement in ganglion cortex (Figs. 1 and 4) adjacent to the exit of dorsal nerve 8.

Figure 1. Horizontal section, terminal ganglion of male *Acheta domestica* showing oblique sections through the left (L) and right (R) medial giant interneurons (MGI). This is a 5- μ m section prepared with the osmium/ethyl gallate method of Wigglesworth (1957). The major branches of the left MGI are indicated in schematic outline, based on cobalt backfills of numerous ganglia. The axon of the left MGI (ax) exits in the left ventral nerve cord. The cell body (cb) is located contralaterally. Sensory axons from the cercal sensilla enter the ganglion via the cercal sensory nerves (csn). The majority of their terminals lie in the glomerulus (g) where they synapse with fine arborizations of the giant interneurons. Scale bar, 100 μ m.

Primary dendritic branches of the MGI were identified in serial sections through dorsomedial regions of the neuropil and were characterized by the following criteria: (a) a location in middorsal levels of the neuropil where cercal sensory nerve and medial giant axon projections were apparent, (b) a markedly large cytoplasmic area, and (c) a distinct bifurcation pattern of the two primary processes. Only those ganglia in which somata and dendrites were bilaterally intact, traceable, and identifiable in serial sections were selected for analysis.

Measurements of ganglion dimensions were made directly through the microscope with a calibrated eyepiece graticule. Grain densities within MGI somata and cell areas were calculated from photographic cross-sections. Intradendritic grain densities were obtained directly through a calibrated eyepiece grid frame on the microscope and mean values were computed from randomly

sampled areas within primary branches of MGI dendrites.

Supplies. New England Nuclear supplied [3 H]*L*-leucine (60 Ci mmol $^{-1}$) and Aquasol. Puromycin dihydrochloride, HEPES, and cycloheximide were from Sigma. All other compounds used were of reagent grade.

Results

Ganglia from S.A._L animals. In agreement with previous findings (Palka and Edwards, 1974; Murphey et al., 1975), long term deafferentation produced a noticeable decrease in terminal ganglion (TG) volume (as measured by cross-sectional area changes) (Fig. 2) when compared to normal (S.C.) ganglia. The volume reduction is due entirely to the loss of the neuropil in the posterior quadrants of the ganglia where the majority of cercal sensory synapses are made. Decreases in TG volume taken from

Figure 2. Whole mount preparations of terminal ganglia. *A*, S.C. (control) ganglion; *B*, S.A._L ganglion (2 weeks following bilateral cercectomy); *C*, S.A._L ganglion (after bilateral cercectomy maintained throughout postembryonic stages); *D*, A.C. ganglion (after chronic deafferentation on the left side). Note marked diminution of the neuropil area in the posterior regions of the S.A._L compared to the S.C. ganglion. The deafferented (*left*) side of the A.C. ganglion shows a decreased posterior neuropil compared to the innervated (*right*) side. The cercal nerve arises from posterior angles of the ganglia in *A* and *B*. They are absent in *C* and present on the *right side* only in *D*. Air-filled tracheae (*t*) show bright white. All magnifications $\times 25$.

both S.A._L male and female animals ranged between 20 and 30%. Measurements of TG dry weight and total protein paralleled the volume changes, with the decreases ranging between 20 and 30%. Cercal sensory nerves of S.A._L ganglia, if present at all, were greatly diminished in size. In contrast, other elements of S.A._L ganglia, including MGI somata and axons, displayed no gross morphological changes with respect to size, relative position, and histological appearance when compared with those of S.C. ganglia. MGI dendrites in S.A._L ganglia appeared qualitatively similar to those in S.C. ganglia though somewhat displaced in position.

Incorporation of [^3H]leucine into S.A._L TG proteins

was depressed significantly compared to the S.C. ganglia (Table IA). When the amount of incorporation was normalized with regard to the loss of TG protein subsequent to deafferentation, the S.A._L ganglia showed a greater than 40% decrease in TCA-insoluble specific activity; TCA-soluble activity was similarly affected. Incorporation of [^3H]leucine into protein was inhibited to a similar degree (85 to 90%) in both groups by 200 $\mu\text{g}/\text{ml}$ of cycloheximide or 100 $\mu\text{g}/\text{ml}$ of puromycin (data not shown).

To examine the possibility that decreased incorporation and uptake might be due to gross differences in extracellular compartments or tissue differences induced

TABLE I
Incorporation of [^3H]leucine into proteins of control and deafferented TGs

Ganglia from S.C., S.A._L, and S.A._s animals were incubated in [^3H]leucine and assessed for protein content and TCA-soluble and TCA-insoluble activity as described under "Materials and Methods." All samples were measured in duplicate.

A. Comparison of control (S.C.) and chronically deafferented (S.A. _L) ganglia				
Ganglion	n	Protein $\mu\text{g} \pm \text{SEM}$	Activity	
			TCA-soluble $\text{cpm } \mu\text{g}^{-1} \text{ protein} \pm \text{SEM}$	TCA-insoluble
S.C.	13	17.1 $\pm$ 1.0	2154 $\pm$ 260	180 $\pm$ 15
S.A. _L	13	11.8 $\pm$ 0.4	1218 $\pm$ 124	106 $\pm$ 15
Difference		-31% ^a	-43% ^a	-41% ^a

B. Comparison of control (S.C.) and short term deafferented (S.A. _s) ganglia				
Ganglion	n	Protein $\mu\text{g} \pm \text{SEM}$	Activity	
			TCA-soluble $\text{cpm } \mu\text{g}^{-1} \text{ protein} \pm \text{SEM}$	TCA-insoluble
S.C.	11	13.1 $\pm$ 1.0	3056 $\pm$ 587	447 $\pm$ 45
S.A. _s	12	12.1 $\pm$ 0.7	3870 $\pm$ 583	515 $\pm$ 43
Difference		-8% ^b	+27% ^b	+15% ^b

^a Statistically significant ($p < 0.001$) by the two-tailed grouped Student's t test.

^b Not significant ($p > 0.10$) by two-tailed grouped Student's t test.

Figure 3. Washout of [^3H]leucine from labeled TGs. TGs were removed from S.C. (○) and S.A._L (●) animals, preincubated for 120 min in oxygenated HBLS containing 100 $\mu\text{g}/\text{ml}$ of puromycin dihydrochloride, and then incubated in a medium containing puromycin and [^3H]leucine (100 $\mu\text{Ci}/\text{ml}$). TGs were removed from the labeled medium, rinsed for 1 min in ice cold unlabeled HBLS, rapidly transferred to large volumes of unlabeled oxygenated medium at 22°C, and maintained for the times shown. Ganglia were assessed for total radioactivity and protein as described under "Materials and Methods." The values shown represent the means of two of each ganglion measured at each time. Standard deviations (not shown) were all within less than 20% of the means.

by deafferentation, a preliminary [^3H]leucine washout experiment was performed (Fig. 3). The slopes of the washout curves were quite similar for the two groups as were the values for half-maximal washout times, suggesting that extracellular metabolite compartments in both groups were generally comparable when the changes in ganglion dry weight or total protein were taken into account. More refined uptake and kinetic studies would be required to determine precisely the basis for changes in amount of incorporation. It appears likely, however, that uptake and incorporation into neural and perhaps glial elements are altered specifically by deafferentation.

Ganglia from S.A._s animals. These TGs had been deafferented immediately following the final stage of postembryonic development and were examined 1 to 2 weeks following the deafferentation. Morphologically, S.A._s ganglia were much nearer in overall appearance to S.C. (control) ganglia than to those of the S.A._L group (Fig. 2). Neuropil volume was only slightly reduced (less than 10%) compared to controls at these times despite atrophy of the cercal sensory nerves. Examination of several S.A._s ganglia prepared for light microscopy revealed notably less diminution of the posterior neuropil than in S.A._L ganglia, even though most, if not all, pre-synaptic terminals had presumably degenerated by this time (see Palka and Edwards, 1974). The changes in TG dry weight and total protein were less in S.A._s than in S.A._L ganglia (also in the 5 to 10% range), similarly reflecting less severe alteration of the TG neuropil.

Incorporation of [^3H]leucine into S.A._s TG protein was not significantly different from that of S.C. ganglia when tested 1 week following deafferentation (Table IB). In fact, compared to control TGs, the S.A._s ganglia displayed slightly higher amounts of uptake and incorporation. The increased incorporation observed at this time probably reflects changes due to a generalized injury response and a correspondingly increased activity of glial cell metabolism. Two weeks following deafferentation, reduction in metabolism was not observed (data not shown), and uptake and incorporation remained similar to that in S.C. ganglia. Aging adult control and denervated groups (i.e., past 2 weeks) began to show signs of general debility and metabolic instability and could not be used reliably for further comparisons at later stages.

Autoradiography of target interneurons in A.C. ganglia. A group of animals was subjected to unilateral (asymmetric) chronic deafferentation and prepared for autoradiographic analysis as described. These A.C. ganglia, when compared to S.C. untreated ganglia, characteristically showed dramatic morphological signs of chronic deafferentation which were limited entirely to the cercectomized side (Fig. 2). The cercal sensory nerve was absent or vestigial and the neuropil volume was greatly reduced on the deafferented side of the A.C. ganglia. Loss of neuropil was largely confined to the posterior quadrant of the TG where most synapses of cercal sensory axons onto target interneuron dendrites are made. The cortical (perikaryal) regions on the treated side of the ganglia, although topographically displaced by the loss of neuropil, appeared qualitatively similar in appearance to the control side and to cortical areas in S.C. ganglia. These findings generally agree with those of

Figure 4. Autoradiography of [^3H]leucine-labeled MGIs in A.C. ganglia. Horizontal 1- μm section (A) through the dorsal region of a labeled A.C. ganglion showing the locations (arrowheads) of somata of control and deafferented (asterisk) MGIs. Magnification $\times 80$. Higher magnifications of control and deafferented somata are shown in B and C, respectively: magnification $\times 800$. Large primary dendritic processes (at the region of bifurcation) of a deafferented MGI are identifiable (arrowhead) in a section of another A.C. ganglion (D) taken at a dorsomedial level. Magnification $\times 80$. Processes of the control MGI were apparent in serial sections obtained from more medially located regions of the ganglion.

previous studies (Palka and Edwards, 1974; Murphey et al., 1975). It was not possible in this study to measure quantitative changes in the growth of giant interneuron dendrites. The primary dendritic processes of MGIs on both sides of A.C. and S.C. ganglia, however, were readily identifiable in serial sections as were the corresponding MGI perikarya in the cortex (Fig. 4). Identification of MGI somata and primary dendrites was based upon the topographical criteria presented by Mendenhall and Murphey (1974), by which individual interneuronal elements can be localized accurately in terminal ganglia.

The distinctive location and size of MGI somata and primary dendrites allowed a quantitative autoradiographic analysis of grain densities of intracellular labeled proteins in 1- μm sections of A.C. and S.C. ganglia (Fig. 4). Direct comparisons of grain densities between paired control and deafferented target MGIs within the same ganglia thus could be made and interanimal variations in [^3H]leucine incorporation could be eliminated.

The results of grain density measurements made in paired MGI somata and primary dendrites from three radiolabeled A.C. and two S.C. ganglia are shown in

Tables II and III. The criteria for valid comparisons of treated and untreated neuropil were the presence in serial sections of intact, identifiable MGI somata and dendrites on both control and deafferented sides. These criteria precluded a large sample size: three from a total of seven A.C. ganglia and two from a total of four S.C. ganglia examined were suitable for quantitation.

It is notable that, in all three A.C. ganglia analyzed, somata of chronically deafferented target MGIs displayed significantly reduced mean grain densities as compared to their paired control somata (Table IIA). The mean decreases in labeled proteins ranged from 16 to 26%; the cumulative mean decrease in the deafferented somata was 22%. In contrast, the cumulative mean grain densities in left and right MGI somata from the two S.C. ganglia examined did not vary significantly from one another (Table IIB).

When intradendritic grain densities were calculated for control and deafferented MGIs, differences were even more pronounced than in somata. It was necessary to make many random measurements over areas of representative primary dendritic cytoplasm. Background grain densities were subtracted due to the overall low labeling densities. The resultant data (Table IIIA) showed that, in all three A.C. ganglia, deafferented MGI dendrites

TABLE II

Incorporation of [³H]leucine into intracellular proteins within MGI somata

Grain densities in MGI somata were quantitated from high magnification photographs of three or four serial 1- μ m cross-sections taken through the central nuclear areas of each soma examined. Cross-sectional areas were measured gravimetrically from the photographs (subtracting nuclear areas) and grains were counted at least twice per section using a calibrated grid overlay. Areas closely adjacent to the glial sheath were excluded from quantitation. Background densities, determined over noncellular areas, represented less than 2% of total somatic labeling.

A. Comparison of grain densities within somata of control and deafferented paired MGIs in unilaterally deafferented (A.C.) ganglia

Ganglion	Mean Grain Density		Mean Difference
	Control MGI Soma	Deafferented MGI Soma	
	<i>grains/100 μm² $\pm$ SD</i>		<i>% $\pm$ SD</i>
X1	90.3 $\pm$ 11.6	76.0 $\pm$ 7.5	-16 $\pm$ 3
X2	50.0 $\pm$ 1.5	38.5 $\pm$ 1.0	-23 $\pm$ 1
X6	47.7 $\pm$ 9.1	35.4 $\pm$ 6.7	-26 $\pm$ 2
Cumulative means $\pm$ SEM	62.7 $\pm$ 11.3	50.0 $\pm$ 10.6	-22 $\pm$ 2 ^a

B. Comparison of grain densities within paired control MGI somata in control (S.C. ganglia)

Ganglion	Mean Grain Density		Mean Difference
	Left control MGI Soma	Right Control MGI Soma	
	<i>grains/100 μm² $\pm$ SD</i>		<i>% $\pm$ SD</i>
C2	66.4 $\pm$ 14.1	72.2 $\pm$ 13.0	+9 $\pm$ 1
C3	52.8 $\pm$ 1.7	45.6 $\pm$ 13.8	-14 $\pm$ 4
Cumulative means $\pm$ SEM	59.6 $\pm$ 5.5	58.9 $\pm$ 10.8	-2 $\pm$ 9 ^b

^a Statistically significant ($p < 0.005$) by the two-tailed paired Student's t test.

^b Not significant ($p > 0.10$) by the two-tailed paired Student's t test.

TABLE III

Incorporation of [³H]leucine into intracellular proteins of MGI primary dendrites

Grain densities within identified MGI dendritic cytoplasm were quantitated directly through a 100- μ m² calibrated microscope eyepiece graticule. A series of 12 independent random measurements were made for each primary dendrite examined in two or three serial 1- μ m sections, resulting in at least 24 density quantitations per dendrite to compensate for labeling variability. Measurements were normalized for background densities (measured over noncellular areas), which represented less than 10% of the dendritic labeling. Grains adjacent to glial layers were not counted.

A. Comparison of grain densities within control and deafferented paired MGI dendrites in unilaterally deafferented (A.C.) ganglia

Ganglion	Mean Grain Density		Mean Difference
	Control MGI Dendrite	Deafferented MGI Dendrite	
	<i>grains/100 μm² $\pm$ SD</i>		<i>% $\pm$ SD</i>
X1	7.58 $\pm$ 3.18	4.75 $\pm$ 2.33	-37 $\pm$ 3
X2	4.67 $\pm$ 2.83	2.20 $\pm$ 1.92	-53 $\pm$ 13
X6	4.05 $\pm$ 1.45	1.47 $\pm$ 1.12	-64 $\pm$ 10
Cumulative means $\pm$ SEM	5.43 $\pm$ 1.08	2.81 $\pm$ 0.99	-51 $\pm$ 7 ^a

B. Comparison of grain densities within paired control MGI dendrites in control (S.C.) ganglia

Ganglion	Mean Grain Density		Mean Difference
	Left Control MGI Dendrite	Right Control MGI Dendrite	
	<i>grains/100 μm² $\pm$ SD</i>		<i>% $\pm$ SD</i>
C2	4.69 $\pm$ 0.56	4.44 $\pm$ 0.72	-5 $\pm$ 15
C3	6.86 $\pm$ 1.86	6.22 $\pm$ 0.84	-9 $\pm$ 6
Cumulative means $\pm$ SEM	5.77 $\pm$ 0.88	5.33 $\pm$ 0.73	-7 $\pm$ 2 ^b

^a Statistically significant ($p < 0.005$) by the two-tailed paired Student's t test.

^b Not significant ($p > 0.10$) by the two-tailed paired Student's t test.

displayed markedly lower grain densities of labeled proteins than did paired control dendrites. Reductions ranged from 37 to 64%; a cumulative mean difference of 51% was found between the paired dendritic processes. Such extreme differences were not observed when grain densities in the left and right MGI primary dendrites in two S.C. ganglia were compared (Table IIIB).

Discussion

We have demonstrated that chronic, but not short term, cercal sensory deafferentation causes a marked depression in [³H]leucine uptake and incorporation into terminal ganglion protein and, most notably, that these decreases in protein synthesis are detected specifically within the somata and large primary dendritic processes of deafferented giant interneurons. Our results also confirm previous findings (Palka and Edwards, 1974; Murphey et al., 1975) that chronic deafferentation induces substantial diminution of the terminal ganglion neuropil volume and extend these observations by showing that short term deafferentation does not lead to such major reductions in the amount of central neuropil. Thus, we find that observed changes in neuropil and target interneuron morphology (Palka and Edwards, 1974; Mur-

phrey et al., 1975) are closely associated with concomitant changes in protein metabolism within these structures following chronic deafferentation. These results lead us to conclude that only deafferentation maintained throughout the critical stages of postembryonic development produces marked change in the growth and metabolism of the giant interneurons and also that removal of presynaptic input per se (e.g., early in adult stages) may not play an important role in the subsequent maintenance of developmentally mature postsynaptic structures. Similarly, loss of presynaptic terminals alone cannot account for the major alterations of neuropil mass observed after chronic deafferentation, since such changes do not occur following short term deafferentation, even though a majority of presynaptic terminals degenerate within 12 hr of cercectomy in *Acheta* (J. S. Edwards, unpublished observations). We therefore propose that intact afferent innervation does play a major role in the growth and development of the postsynaptic dendritic processes, probably by influencing the general level of metabolism within the giant interneurons via as yet undefined trans-synaptic mechanisms.

The large overall decrease in [^3H]leucine uptake and incorporation into ganglion proteins observed following chronic deafferentation suggests that many central elements in the ganglion are probably affected, directly or indirectly, by removal of cercal afferents. It is likely that, aside from MGI, other giant interneurons that are innervated by cercal afferents and which may undergo retardation of growth following chronic deafferentation (Murphy et al., 1975; Murphy and Levine, 1980) also might show corresponding decreases in [^3H]leucine metabolism. Additionally, smaller interneurons that may be part of this neural network could be similarly influenced by deafferentation. We do not consider, however, the observed decreases in uptake and incorporation into ganglion proteins following chronic deafferentation representative of a generalized injury response to deafferentation. Most invertebrate and vertebrate neurons respond to denervation by manifesting an increased rate of protein synthesis (Watson, 1974; Denburg and Hood, 1977; Meyer and Bittner, 1978b) or display striking morphological stability in response to such manipulations (Tweedle et al., 1973; Clark, 1977; Krasne and Lee, 1977). In addition, we noted no evidence of increased glial hypertrophy, connective tissue proliferation, or obvious loss of CNS neurons (M. R. Meyer and J. S. Edwards, unpublished observations) following deafferentation.

To our knowledge, this is the first study to show that removal of presynaptic input from known postsynaptic target neurons can induce detectable decreases in amino acid uptake and incorporation within somata and large dendritic processes. Although our studies do not address directly the means by which the level of postsynaptic cell metabolism is influenced by the presence of afferent innervation during postembryonic development, any of several possible mechanisms may be involved. These include direct transneuronal "trophic" effects, generalized interactions influencing the overall level of MGI metabolism, and nonspecific effects arising from loss of afferent innervation.

Since giant interneuron dendrites are located on neurites rather far removed from perikaryal sources of pro-

tein synthesis, they may be dependent upon extraneuronal (e.g., presynaptic, glial) elements for some of their metabolic requirements, especially during periods of extensive growth and development. For example, as the number of presynaptic inputs increases during development, such inputs may contribute to correspondingly greater direct exchange of metabolites with their targets. It has been demonstrated that extrinsic sources may play active roles in the trophic maintenance of other invertebrate neurons (Sarne et al., 1976; Meyer and Bittner, 1978a, b) mediated by direct intercellular transfer of metabolites into neurons and active exchange of molecules between neurons and glia (Lasek et al., 1974; Gainer et al., 1977; Meyer and Bittner, 1978b). Evidence favoring a metabolic inductive effect of presynaptic upon developing postsynaptic neurons has been described (Black et al., 1971; Black, 1978).

An alternate mechanism to direct transfer of metabolites would entail an indirect regulatory role for presynaptic inputs whereby the presence of increasing numbers of terminals on the dendrites could serve as a chemical signal to the neuronal perikaryon to synthesize and transport greater amounts of the proteins necessary for dendritic growth and development. This process might involve the retrograde transport of metabolic cues from the dendrites to the perikaryon and the subsequent increased synthesis and somatofugal shipment of macromolecules to the dendrites. Retrograde intraneuronal transport has been observed in a wide variety of neural systems and may subserve mechanisms regulating neuronal growth and differentiation (Stöckel and Thoenen, 1975; Schwartz, 1979).

It is also possible that the decreased incorporation of [^3H]leucine observed following chronic deafferentation of MGI (and presumably other postsynaptic target neurons) occurs indirectly as a consequence of diminished growth and elaboration of postsynaptic dendritic arborizations. That is, removal of a large portion of normal afferent input during critical developmental periods results in less growth of postsynaptic elements and subsequently less demand for protein synthesis within the deafferented neuron. Thus, although decreased protein metabolism would be secondary to decreased dendritic growth, an unspecified type of regulatory interaction between pre- and postsynaptic elements would still occur. Such an indirect mechanism would be in agreement with the finding that [^3H]leucine incorporation is not decreased following short term deafferentation of fully developed postsynaptic targets.

It remains unclear why different vertebrate and invertebrate neurons can display such a wide variety of responses to deafferentation or denervation, ranging from massive degeneration and cell death to little, if any, observable consequence. Our results, however, strongly reinforce the recurrent theme that neurons appear most susceptible to the effects of denervation at early stages of development (Smith, 1977). In addition, the concept that invertebrate neurons are significantly different from vertebrate neurons in their apparent resistance to the effects of denervation should be interpreted cautiously, particularly with respect to early developmental stages of growth.

Little is known of the nature of the proteins involved

in the growth and development of giant interneurons, their mode of regulation, or their physiological significance. However, current investigations (M. R. Meyer and J. S. Edwards, manuscript in preparation) have shown that chronic, but not short term, deafferentation markedly reduces the density of putative cholinergic receptor sites in the terminal ganglion (Meyer and Edwards, 1980). Such receptor macromolecules are thought to be located at cercal sensory-giant interneuron synapses and thus may represent a major class of membrane surface molecules that are regulated developmentally by the presence of sensory nerve terminals and which are important in the synaptic function of the giant interneuron.

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