REGENERATION OF AN IDENTIFIED CENTRAL NEURON IN THE CRICKET

I. Control of Sprouting from Soma, Dendrites, and Axon¹

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Abstract

When the axon of the medial giant interneuron (MGI) of the cricket is axotomized close to the cell body, the normally stable, characteristic dendritic arborization is induced to sprout supernumerary neurites. The origin of the induced dendritic sprouts is not random; they emerge preferentially from the dendritic tips and branches close to the exit of the axon from the terminal ganglion. If any growth also occurred from the axon, there was a reciprocal relationship between the extent of dendritic and axonal sprouting. On the other hand, axotomy distant to the cell body induces sprouting only from the axon and does not alter the dendritic structure of the MGI.

After crushing the ventral nerve cord distant to the cell body, the MGI sprouted neurites from the proximal axonal stump which crossed the site of lesion and continued growing within the distal cord. After a distant cut of the cord, however, the axonal neurites formed a neuroma in the proximal cord stump at the site of lesion and stopped elongating after 1 month. At this time, supernumerary sprouts first began to emerge from the normally smooth, rounded contours of the cell body.

Based on these observations, we propose that axotomized neurons produce membrane at a constant rate. This newly synthesized membrane is preferentially inserted into neurites emerging from the proximal axonal stump. When axonal neurites stop growing in a neuroma following a distant cut, then this new membrane appears as supernumerary neurites from the soma. After a close cut, the axon often dies back into the ganglion and appears unable to receive the full complement of sprouting membrane. In such cases, the balance of the newly synthesized membrane is inserted into the dendrites and the characteristic structure of the arborization is significantly altered.

Differential growth in discrete regions of a developing neuron eventually results in a fully differentiated nerve cell with three morphological compartments: the soma, the dendrites, and the axon. This structural specialization may arise during development from a selective distribution pattern of specific membrane components characteristic of each neuronal compartment. The distinct structural and functional properties that emerge in each compartment during development are stabilized and maintained to yield the mature neuron.

Axotomy can be employed to study the insertion of newly synthesized membrane associated with the induced regenerative growth. In such an experimental framework, a mature, fully developed neuron is challenged by partial removal of a specific morphological compartment, namely, its axon. To restore its fully differentiated state, an injured neuron must specifically initiate axonal growth while retaining the mature properties of the undamaged dendrites and cell body. Sprouting induced by axotomy therefore provides a model system for studying the mechanisms controlling both regional growth and stabilization of structure and function in a single neuron.

We have chosen to investigate regeneration of an injured, identified central neuron in an insect to study neuronal growth and differentiation. In the adult cricket central nervous system, interneurons can be induced to sprout new neurites from the axon as well as from the cell body and the dendrites. In this study, we address two

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specific questions. What determines the location of new sprouts in a neuron after axotomy? What regulates the extent of growth in a particular region of a neuron?

The giant fiber system of the cricket, in particular the medial giant interneuron (MGI), was chosen as a suitable model for studying regeneration because it permits an assessment of the induced growth and change in electrical properties following axotomy in a single, identified cell. The MGI in the cricket has a well defined dendritic arborization (Mendenhall and Murphey, 1974), specific synaptic connectivity with sensory afferents (Matsumoto and Murphey, 1977), and a characteristic electrophysiological behavior (Murphey et al., 1977). In addition, it has been extensively investigated throughout development by Murphey and co-workers (Murphey et al., 1975, 1976; Murphey and Levine, 1980). All parts of this neuron can usually be identified morphologically, whether they are in the neuropil, the cell body layer, or at the axon terminal. It is thus possible to correlate directly events in different cellular compartments of one neuron, i.e., between the regenerating neurites at the axon end and the concomitant changes in the cell body and dendrites of the same cell.

In this investigation, the adult MGI was induced to sprout neurites from the axon, dendrites, and cell body by cutting or crushing the axon at various distances from the cell body. A distant cut of the axon (at 2000 μm or more from the terminal ganglion containing the cell body) initially elicits sprouting from the proximal axon stump and then, at later times, from the cell body. With this distant lesion the dendrites remain morphologically stable. On the other hand, a close cut of the axon (at 100 to 200 μm from the terminal ganglion) causes sprouting in the dendritic compartment. Axonal outgrowth in this case is usually reduced, while the cell body remains morphologically stable.

Several factors were found to govern the sprouting response to axotomy. Following distant axotomy, sprouting occurs preferentially at the proximal axon stump. After a crush, such axonal sprouts regenerate across the site of lesion and continue to grow linearly within the distal connective, often passing through several abdominal ganglia. However, after a cut, axonal sprouts grew only for a limited time and remained confined to the site of lesion, forming a neuroma. In such cases, after the axonal neurites had ceased growth, supernumerary sprouts were observed to emerge from the cell body.

Following a close cut, the proximal axonal stump degenerated in a retrograde direction for a significant distance, occasionally well into the neuropil of the terminal ganglion. When this occurred, axonal sprouting was less profuse, often even totally absent. In these cases, supernumerary sprouts emerged from the characteristic dendritic arborization of the MGI. These sprouts grow linearly throughout the neuropil and may even enter peripheral nerves and central connectives never occupied normally by the intact, parent MGI arborization. We show that the amount of sprouting from the dendritic compartment is inversely proportional to the extent of sprouting from the axonal stump. Moreover, the dendritic sprouts do not originate randomly from any point on the MGI arborization, but are more likely to emerge from

the tips of the large dendrites and from collaterals near the exit of the axon from the ganglion.

Materials and Methods

Four-week-old, immature crickets, *Acheta domesticus*, acquired from Fluker's Cricket Farm (Baton Rouge, LA), were kept in crowded colonies at 30 to 32°C on a 14-/10-hr light/dark cycle and fed daily with lettuce and dried dog food. Only males that had molted to their adult stage overnight were used for subsequent experiments.

Operations. One-day-old adult males were anesthetized by cold (4°C) and were kept at this temperature during surgery by placing them on a platform cooled by a Peltier unit. The animal was fastened ventral side up, and the ventral abdominal surface was cleaned with a 0.01% solution of Zephiran Chloride, rinsed in sterile saline, and dried. A rectangular aperture was cut into the abdominal exoskeleton over the appropriate location on the ventral nerve cord and the flap was folded back. One of the exposed interganglionic connectives was either cut with iridectomy scissors or crushed with fine forceps at the desired distance from the terminal ganglion indicated as sites t_1 to t_5 in Figure 1. The flap of cuticle was folded back over the wound, and a small piece of sterile tissue paper was placed over it to hold it in place and prevent excess bleeding. Operated crickets recovered in 10 min at room temperature and then were kept individually in plastic boxes at 30 to 32°C. Over 95% of the animals survived until they were sacrificed.

Electrophysiology. Crickets were anesthetized by cold (4°C) prior to dissection. A dorsal midline cut along the full length of the animal permitted the cuticle to be pinned apart. The animal was eviscerated and the resultant body cavity was filled with cricket saline (Usherwood and Grundfest, 1965). The terminal ganglion was prepared for recording by placing it on a support intro-

Figure 1. Drawing showing the posterior portion of the central nervous system of the cricket. The terminal ganglion (A_5) contains the somata and dendritic arborization of the giant interneurons. The right medial giant interneuron (MGI) is shown with the cell body located contralateral to the axon. Afferents from the sensory receptors on the cerci enter the ganglion via the cercal nerves (cn) and synapse on the dendrites of the interneurons. Each interneuron projects one large axon anteriorly into an interganglionic connective (IGC), passing through all abdominal ganglia (A_4 to A_1). The axons give rise to short, stubby collaterals in each ganglion. Intracellular records were obtained from the MGI soma. The sites of close axotomies are shown as t_1 and t_2 , while distant axotomies are indicated as lesion sites t_3 to t_5 . Approximate distance of each lesion site from the terminal ganglion is: $t_1 = 200 \mu\text{m}$; $t_2 = 400$ to $500 \mu\text{m}$; $t_3 = 2.0 \text{ mm}$; $t_4 = 3.3 \text{ mm}$; $t_5 = 4.6 \text{ mm}$.

duced beneath. With this procedure no nerves or tracheoles entering the ganglion were cut. For intracellular recordings from the MGI soma located on the lateral edge of the ganglion, the support was used to tilt the terminal ganglion about 45° laterally to permit tangential access to the soma by a microelectrode. All experiments were carried out at room temperature (22°C).

Microelectrodes filled with the dye Lucifer Yellow (Stewart, 1978) were used for intracellular staining. Axons or cell bodies were penetrated with these electrodes and the dye was iontophoresed with hyperpolarizing current pulses of 1 Hz, 500 msec duration, and 20 nA amplitude for 5 to 10 min.

Morphology. Dye-filled interneurons were allowed to stand for 30 min to 2 hr before being fixed and processed for whole mount viewing (Stewart, 1978). These whole mounts were placed into depression slides in methyl salicylate, coverslipped, and photographed under transmission fluorescence optics at successive focal planes. Drawings of the MGI arborization were prepared by projecting the resulting slides on white paper sheets at a fixed distance from the projector lens.

When sections for microscopy were desired from a specific whole mount preparation, the cleared tissue was taken back to 100% ethyl alcohol (two changes for 10 min each) and then was prepared by conventional techniques (Wood et al., 1977) for embedding in Spurr's medium. Five-micrometer sections were cut on an LKB microtome and photographed using epifluorescence optics.

Although these whole mount preparations of neurons stained with Lucifer Yellow provided good sections for low magnification light microscopy, the formaldehyde fixation was not adequate for the examination of cytological detail. Therefore, ganglia from experimental animals were also fixed using a protocol commonly employed for electron microscopic study of insect nervous tissue (Wood et al., 1977). These tissues were similarly sectioned, stained with 0.5% toluidine blue in 1% borax, and subsequently examined and photographed under phase contrast optics.

Results

Neuritic outgrowth induced by close axotomy

Dendritic outgrowths. Close axotomy of the MGI, achieved by cutting the ventral nerve cord within 200 μ m of the terminal ganglion, evoked pronounced sprouting from the identified dendritic tree (Fig. 2). The dendritic branching pattern of the normal MGI (Fig. 2a) is well defined and stereotyped (Mendenhall and Murphey, 1974), such that any change in gross dendritic morphology is readily detectable. New neurites can be seen emerging as early as 2 days after such close axotomy, and they continue growing throughout the post-axotomy period as late as 3 weeks after transection.

Most neurites sprouting from an axotomized dendritic tree take tortuous paths throughout the neuropil of the terminal ganglion, twisting and turning upon themselves. Many of these sprouts have simple swellings at their ends, or swellings with branchlets, similar to growth cones. The induced dendritic neurites can grow into

nerves entering or leaving the terminal ganglion, such as the cercal nerve or the central connectives. Within a few days post-axotomy, many neurites have already reached lengths of hundreds of micrometers, developed complex branching patterns, and invaded regions of the nervous system never occupied by normal MGI arborizations.

Eleven control cells contralateral to such close cut MGIs displayed completely normal dendritic arborizations up to 3 weeks after injury. It is clear, therefore, that the dendritic sprouting is induced directly by the lesion and does not result from systemic influences evoked by the injury.

A lesion of the ventral nerve cord axotomizes other identified giant interneurons (Mendenhall and Murphey, 1974) in addition to the MGI. Some of the interneurons, including the lateral giant interneuron (LGI), were injected with dye at equivalent times following a close cut. Supernumerary neurites also emerged from the identified dendritic arborizations of these cells. Such dendritic neurites were similar to those induced in the MGI, in that they coursed throughout the neuropil and often grew into sensory nerves or the central connectives.

Site of origin of dendritic sprouts. Although supernumerary sprouts emerged from almost any point on the dendritic tree of axotomized MGIs, examination of many such preparations revealed that the probability of a sprout emerging from certain locations was higher than for others. Figure 3 shows the origin of sprouts pooled from 71 preparations. The number of observations of sprouts originating from each site are marked on the schematic drawing of the dendritic tree. The ends of the primary dendrites and the short branches near the origin of the axon in the terminal ganglion more often gave rise to supernumerary sprouts than at any other location. Sprouts arose less frequently from branch points and smaller dendrites. Only a few preparations showed sprouts from the initial neurite, or the process connecting left and right dendritic branches. It is clear that sprouting does not arise randomly from any point on the dendritic tree of an axotomized MGI, but originates preferentially from the ends of the primary dendrites and the branches close to where the axon leaves the ganglion.

Dendritic versus axonal outgrowth. A reciprocal relationship was found between the extent of axonal and dendritic sprouting among MGIs lesioned close to the terminal ganglion. This is illustrated in Figure 4, which shows four representative MGIs 8 days after axotomy at t_1 . Figure 4a is an example of the extreme case in which the axon sprouted a large, complex arborization at the site of lesion. The parent dendritic tree resembles that of a normal MGI. Figure 4b shows a small increment in dendritic growth represented by several supernumerary neurites on the parent arborization. This is accompanied by a reciprocal decrease in axonal sprouting as seen by comparison to Figure 4a. The MGI in Figure 4c shows another incremental shift in the relationship between axonal and dendritic growth. Less axonal arborization than that shown by *b* or *a* results in more pronounced dendritic sprouting at the same postoperative time. Finally, in Figure 4d the MGI has generated only small, underdeveloped axonal sprouts but has produced extensive neuritic growth from the dendrites. Among these

Figure 2. Whole mount preparations of MGIs injected with Lucifer Yellow. *a*, Normal dendritic arborization in the terminal ganglion. *b* to *d*, Examples of dendritic supernumerary sprouting (arrows) emerging from the MGI arborization in the terminal ganglion at 6 (*b*), 10 (*c*) and 14 (*d*) days after lesion of the axon at t_1 .

Figure 3. Summary diagram derived from 71 preparations with supernumerary dendritic sprouts. The number opposite each solid circle indicates the number of preparations showing sprouting at that point. Each outgrowth of at least 50 μm in length was scored. Note that sprouts most frequently arose from the tips of the two large dendrites and from branches where the axon exits the ganglion.

four cases, this is the neuron which has given rise to the greatest extent of dendritic supernumerary sprouting. This series suggests that, in the interval of the 8 days since lesion, the respective MGIs have generated approximately equivalent amounts of sprouting membrane. However, the distribution of this new membrane varied. In one extreme case, *a*, the majority was apportioned to the axonal compartment, whereas in the other extreme, *d*, the majority was channeled into the dendritic compartment. Intermediate points on this spectrum of membrane apportionment are represented by *b* and *c* in Figure 4.

At later times post-axotomy, the extremes in this membrane apportionment become exaggerated as more total membrane is inserted into the arborization. In Figure 5, *a* and *b*, an MGI pair is shown at about 12 days after cutting their axons at t_1 . The neuron in *a* represents that case in which the axon received most of the new sprouting membrane and therefore developed a large, complex arborization at its end. In contrast, the MGI in *b* has less axonal growth than the one in *a*, but has generated many neurites from its dendritic tree. These have grown in all directions, including one dendritic sprout which entered the connective and others which grew into the cercal nerves.

Crush of the axon close to the terminal ganglion will also elicit dendritic sprouting. Figure 5, *c* and *d*, represents two MGIs 13 and 15 days, respectively, after a close crush at site t_1 . The induced axonal outgrowth in *c* is extensive, with several neurites growing across the site of lesion into the distal connective. This neuron has only two new, small dendritic sprouts. The axon of the MGI in *d*, however, has died back well into the terminal ganglion and no axonal outgrowth at all has been produced. The dendrites, on the other hand, have received the full complement of the sprouting membrane. Two sprouting neurites from the dendritic tree have made their way into the site of lesion, branched there, and grown into the distal connective, analogous to the behavior of the axonal neurites in Figure 5*c*.

Although induced dendritic sprouts were most often observed after a cut at t_1 , the extent of sprouting was not determined purely by the location of the cut; i.e., not all MGIs axotomized at t_1 displayed the same amount of dendritic sprouting. In addition, 3 of 14 MGIs cut at a more distant location, t_2 , also displayed dendritic sprouts. The apparent variability in the morphological response of the dendrites can be resolved if one proposes that dendritic sprouting is not determined solely by the location of the axotomy, but also by the amount of retrograde degeneration (axon die-back) of the proximal stump. Indeed, the extent of proximal axon die-back varied among numerous preparations, despite the fact that each nerve cord was lesioned at the same location. Such variability in the extent of axon die-back has been reported in single, central neurons following lesions of the lamprey spinal cord (Roederer et al., 1983).

We can explain the variable extent of dendritic sprouting by comparing it with the amount of axonal growth in the same neuron. We suppose that minimal axon die-back allows a longer, healthier stump to survive and that this is conducive to axonal sprouting from the end of this proximal stump. On the other hand, if die-back is pronounced, as when the proximal axon segment degenerates well into the neuropil, little or no axonal sprouting arises from such a stump. Hence, in an MGI which possesses a relatively healthy axon remnant with profuse sprouting from the proximal stump (e.g., Figs. 4*a* and 5, *a* and *c*), little or no dendritic sprouting is seen. On the other hand, an MGI with a greater extent of proximal axon die-back (e.g., Fig. 5*d*) and, therefore, sparse axonal sprouting will show profuse growth from its dendritic compartment.

In summary, it is clear that close axotomy induces sprouting from the MGI. However, the sprouting membrane is apportioned in a reciprocal relationship between neurites growing from the dendritic and axonal compartments. The length and the metabolic state of the surviving axon stump may be important factors in determining the location of the new sprouts.

Responses to distant axotomy

Axonal regeneration. Following axotomy of the ventral nerve cord at greater distances from the terminal ganglion (sites t_3 , t_4 , t_5 shown in Fig. 1), the MGI produced three to four sprouts from the proximal axonal end

Figure 4. Drawings of four MGIs at 8 days after axotomy at t_1 . In all cases the parent remnant of the injured neuron is shown in *solid black*, while the new sprouts are shown as *white, unfilled processes*. *a*, An instance in which all of the outgrowth occurred at the axon terminal, while the parent MGI arborization remained structurally stable. *b*, Another MGI, with the same type of lesion, demonstrates comparatively less axonal outgrowth than in *a*. Note, however, the presence of some induced sprouts on the dendritic arborization. *c*, This MGI has even less axonal outgrowth and comparatively more dendritic sprouts. Finally, in *d*, the major portion of outgrowth has occurred in the dendritic compartment, with very little sprouting from the axonal end.

within the first 6 days (Fig. 6*a*). If the ventral nerve cord was lesioned by a cut, then the sprouts remained confined to the area of the lesion, growing into a tangled mass, termed a neuroma (Fig. 6, *b* and *c*). By 3 to 4 weeks, the sprouts in this neuroma ceased elongating and no net growth was observed beyond this point up to 72 days following the cut. If, on the other hand, the ventral nerve

cord was lesioned by a crush, then the sprouts from the proximal axonal stump of the MGI traversed the lesion into the distal connective. They grew continuously in a linear manner within the nerve cord, often traversing several abdominal ganglia. Within the neuropil of such abdominal ganglia (Fig. 6*d*), the growing neurites were observed to form branches and collaterals, often much

Figure 5. Drawings of MGIs after close axotomy. New growth is indicated as in Figure 4. *a* and *b*, Two MGIs at 12 (*a*) and 11 (*b*) days after a cut at t_1 . In *a*, note that all of the outgrowth has occurred in the axonal compartment (*white processes*) while the parent dendritic arborization has remained stable. By comparison, at an equivalent period of time following the same type of lesion, *b* shows less axonal sprouting while many dendritic sprouts have emerged from the parent arborization. This includes one dendritic sprout which has grown parallel to the axon into the neurons at the end of the cut connective. *c* and *d*, Two MGIs after a crush lesion at site t_1 , 15 (*c*) and 13 (*d*) days later. Note that the MGI in *c* has given rise to axonal sprouts which grow past the site of lesion (*arrow*) into the distal connective. The axon of the MGI in *d* has died back almost completely into the ganglion (*long thin arrow*) far from the site of lesion (*short arrow*). Note the proliferation of dendritic outgrowth. Some of these exit the ganglion through the connective, growing into the site of lesion and even into the distal connective, much like the axonal outgrowths in *c*.

Figure 6. *a*, MGI proximal axonal segment showing cut end 6 days after transection. *b*, MGI axon end 10 days after cut. *c*, MGI axon end 15 days after cut. *d*, Simultaneous fills of MGI and LGI, 47 days after a crush of the connective at the location indicated by the *arrow*. Note that neurites from both axons have traversed the site of lesion and have entered the adjacent, more rostral abdominal ganglion, where they have branched. Calibration is the same for all figures.

more profusely than the short, stubby collaterals emerging from normal MGI axons.

In summary, distant axotomy of the MGI axon always elicits sprouting from the proximal axon stump. Following a cut, the axonal sprouts within the neuroma at the site of lesion cease elongating by 3 to 4 weeks. After a crush, the emerging neurites traverse the site of lesion and continue growing within the distal nerve cord.

Dendritic stability. Unlike the profuse sprouting elicited by close cut, the dendrites of the MGI remained morphologically stable following axotomy at greater distances from the terminal ganglion (t_3 to t_5 in Fig. 1). Figure 7 shows pairs of homologous MGI neurons in whole mount, one of which was severed at t_3 , while the contralateral MGI served as control. It is apparent that, at the level of the primary dendritic branches, no change has occurred either at 7, 21, or 54 days following axotomy. No major detectable alterations of the dendritic arborization, either in terms of the total length of individual branches or the complexity of secondary structure were observed up to 72 days post-axotomy.

To quantify the stability of dendrites to axotomy, the lateral and medial dendrites were measured in length and compared in pairs of MGIs in 11 preparations. One MGI in each preparation was axotomized at t_3 or t_4 , while the contralateral MGI remained intact and served as control. These preparations covered a range of 4 to 57 days post-axotomy. The ratio of dendrite lengths of the axotomized to the control MGI was not significantly different from unity (average values: 0.96 ± 0.08 for the medial dendrites, 0.99 ± 0.10 for the lateral dendrites) over this period. In addition, 23 other distantly axotomized MGIs whose control side homologue had not been filled in the same preparation were examined, and their primary dendrites were indistinguishable from normals at all times.

All impaled MGI axons, control and axotomized, responded electrophysiologically to wind puffs directed to the left or right cercus. Therefore, at least a minimum number of sensory inputs, sufficient to permit synaptic transmission, remained at all times following distant axotomy.

Soma outgrowths. Although the dendrites of MGIs remained morphologically stable following a distant cut, we observed that about 5 weeks after cutting their axons, many interneuron somata, including the MGI, sprouted supernumerary outgrowths. Figure 8*a* shows a cell body of interneuron 9-3 (nomenclature of Mendenhall and Murphey, 1974) whose axon was severed 47 days before. Numerous outgrowths and surface irregularities can be seen on this soma, which is normally smooth and round. Soma abnormalities were not observed at the whole mount level at times earlier than 24 days postoperative, nor were they seen in preparations in which the connective had been crushed instead of cut. Control side interneuron somata appeared normal at all times. The preparation shown in whole mount (Fig. 8*a*) was embedded and sectioned to examine the soma outgrowths at higher magnification. Figure 8, *b* to *d*, shows consecutive sections through this soma, indicating a proliferation of neurite-like extensions from the surface of the cell body induced by axotomy.

Whole mount preparations of interneurons stained with Lucifer Yellow are necessarily fixed in formaldehyde (Stewart, 1978) which causes some distortion of the tissue. Therefore, a series of axotomized animals was prepared by a conventional protocol using glutaraldehyde and osmium tetroxide (Wood et al., 1977), which provides excellent fixation of insect central neurons for electron microscopy. Fifty-two ganglia, ranging from 5 to 66 days post-transection, were serially sectioned at 3 μ m thickness. The cell bodies of various identified giant interneurons were examined, comparing the axotomized member with its control side homologue in the same ganglion.

Figure 9 shows a comparison of the axotomized and intact control LGI cell bodies in the same ganglion 60 days after unilateral transection. Normally, the cell body is rounded and bears only one neurite, commonly known as the initial neurite, connecting the soma to the arborization in the neuropil. However, axotomized somata displayed one or more supernumerary neurites emerging from the soma surface. In addition, their contours were no longer rounded but bore numerous irregularities and projections. Soma outgrowths often grew to 100 μ m in length and displayed a pale cytoplasm (Fig. 9).

At 35 days or more after distant axotomy, 27 of 37 MGI cell bodies bore neuritic outgrowths or other smaller projections from their normally rounded perimeters. The contralateral, intact MGI somata never showed such features and appeared indistinguishable from normals. The earliest detectable outgrowths were found on a soma 24 days after axotomy, but usually, these surface projections were not seen on axotomized cell bodies until about day 35.

Discussion

Dendritic stability following distant axotomy. Following a distant axotomy, whether by cut or by crush, the dendrites of the MGI remain unaltered and morphologically stable. This occurs at a time when the proximal axonal stump is engaged in vigorous sprouting. There is a considerable variability among different species and neuron types in terms of the dendritic reaction to axotomy. It is generally believed that vertebrate dendrites display some morphological lability in response to axotomy. This involves an initial retraction followed by a re-expansion of dendritic length when the regenerating axon forms synaptic connections with its target (Sumner and Watson, 1971; Fishman and Cohen, 1974; Purves, 1975). In several arthropods the dendrites remain remarkably stable following axotomy (Tweedle et al., 1973; Horridge and Burrows, 1974; Kuwada and Wine, 1981). However, in the snail (Bulloch et al., 1980; Bulloch and Kater, 1981) and in some neurons of the leech (Muller and Carbonetto, 1979), dendrites of specific neurons alter their branching patterns and, in the case of the snail, also their connectivity in the neuropil following axotomy. Even dendrites of motoneurons in the roach, which are stable to simple axotomy, can be induced to sprout supernumerary neurites by massive deafferentation of the ganglion in addition to axotomy (Pitman and Rand, 1982).

In the cricket, the dendritic arborizations of identified giant interneurons remain morphologically stable after

Figure 7. Whole mount preparations of both MGIs in each ganglion, one cut (marked X) at t_1 , and the other left intact. Postoperative times are 4 (a), 24 (b), and 51 (c) days. Note that the major dendrites of the cut MGI resemble those of the control cell.

Figure 8. *a*, Whole mount of dye-filled interneuron designated 9-3, at 47 days after cut at location t_1 . The *arrow* indicates the cell body, which has given rise to numerous neuritic outgrowths. *b* to *d*, Consecutive sections through the cell body in *a* viewed in epifluorescence optics showing the nature of the neuritic outgrowth from the soma in greater detail.

distant axotomy up to 74 days following a lesion. Therefore, the dendritic stability of these neurons resembles that seen in many invertebrate neurons following axon section. Induced sprouting occurs preferentially at the proximal end of the axon following both distant cut or crush, without affecting the morphology of the dendritic branches in the same interneuron.

Sprouting from the soma membrane induced by distant axotomy. Distant axotomy of the MGI always caused initial sprouting from the proximal axon end. In certain cases, it also evoked sprouting from the cell body beginning about 35 days post-transection. The time of initiation of sprouts from the soma coincided with the time at which axonal neurites, confined to a neuroma at the proximal stump, had ceased growing. A possible interpretation of induced sprouting from the soma in the

cricket is that axotomized MGIs produce new membrane components at a constant rate following lesion. After a crush, such new membrane is preferentially and continually inserted into the elongating axonal neurites growing across the lesion into the distal connectives. Initially, after a cut, new membrane is also inserted preferentially into the axonal neurites growing within the neuroma. However, the linear growth of axonal neurites growing in such neuromas stops at about 1 month after lesion. This is also the time when the supernumerary outgrowths are first observed on the corresponding somata. Therefore, it is possible that at this later time (1 month after lesion) newly synthesized membrane components can no longer be inserted at the axon end and therefore are preferentially channeled into the soma instead. The fact that somata of interneurons axotomized by crush, which

CONTROL SOMA

AXOTOMIZED SOMA

Figure 9. Representative section through the intact LGI soma (control) and consecutive sections through the homologous LGI soma axotomized 60 days previously at site t_1 . Arrows indicate a long, supernumerary neurite that extends from the axotomized soma (S) and courses for approximately 100 μ m beneath the ganglion sheath.

had continually regenerating axonal neurites, did not sprout lends further support to this interpretation.

Dendrites can be induced to sprout by close axotomy. Axotomizing the MGI close to the terminal ganglion evoked a different growth response in the injured neuron. Vigorous dendritic sprouting was observed in many cases, particularly when the axon had either died back into the neuropil or given rise to only a small terminal arborization. Hence, it is clear that the response of the MGI dendrites to axotomy varies; it depends critically upon the proximity of the axonal lesion to the soma, and the extent of the subsequent axonal sprouting.

A significant variable correlated with the distance of the lesion from the soma may be the length of the remaining proximal axon stump. The close lesion is frequently associated with such an extensive retrograde degeneration (die-back) of the proximal axon stump that it essentially disappears. When this happens, profuse growth from the dendrites invariably occurs. In this respect, it is important to note the observation of Muller and Carbonetto (1979) on the leech indicating that the axotomized S cells with dendritic sprouts were precisely

those that had "withdrawn" their axons. Similarly, in order to elicit the sprouting response from snail neurons, the connective bearing their axons must be crushed within 100 μ m of its origin (Bullock and Kater, 1981). The published intracellular dye injections of such neurons point to the absence of the original axon stump, suggesting its retrograde degeneration. Instead, many small regenerating neurites emerge from the dendritic arborization and enter the nerve formerly occupied by the axon.

The nature of dendritic sprouts. During development, growth and elongation of a given dendritic branch is stimulated by the addition of new afferent synapses upon it (Kimmel et al., 1977; Murphey et al., 1975, 1976). In this respect, it would be of interest to know whether the dendritic sprouts resulting from close axotomy in the adult MGI also form synapses as they elongate; i.e., is the dendritic growth induced by close axotomy similar to dendritic growth during development in that it is stimulated by the formation of new synaptic contacts? The linearity of the new growth and the lack of any specialized structures along their length suggest that this is not the case. Rather, some features of the dendritic outgrowth from axotomized MGIs suggest that they resemble instead the growth of axonal neurites during regeneration. For instance, the induced sprouts originating from the MGI dendrites often displayed growth cone-like swellings at their ends, which frequently gave rise to small microspikes. In many cases, these neurites were observed to grow linearly within peripheral nerves and the connectives. Thus they resemble more the behavior of regenerating sprouts originating from the proximal axonal stump. Both axonal and dendritic sprouts grow in a straight line within connective tracts, but branch and turn when entering the less isotropic cellular architecture in the neuropil of an abdominal ganglion or of the neuromas. Hence, neurites originating from two different anatomical compartments of a neuron display similar behavior during induced growth. This suggests that at these early stages of induced outgrowth, the same properties govern the controlled insertion of membrane in growing neurites originating from dendrites and axons.

Control of dendritic sprouting. It has been proposed that sprouting is the result of an inherent tendency of a neuron to maintain a determined cell volume (Schneider, 1973). This has been demonstrated in the development of axonal arborizations in the optic tract (Schneider, 1973) and the lateral olfactory tract of newborn hamsters (Devor, 1975). Schneider reported that if one terminal area of the optic tract was removed, then growing fibers from that tract branched much more densely in the remaining intact target areas and also invaded regions not normally occupied by optic nerve terminals. Schneider attributed this increased branching to a "pruning" effect, whereby removal of part of an axon arborization accelerates growth of the remaining complement. However, a difficulty encountered in these studies is that of interpreting the behavior of individual axonal branches from the observations on central tracts containing thousands of axons.

The reciprocal relation between dendritic and axonal sprouting following close cuts of the MGI illustrates the

principle of conservation of total sprouting at the level of one identified cell. It represents the first reported case indicating control over the sprouting occurring simultaneously in two different neuronal compartments, the dendrites and the axon. When axonal branching of the MGI was profuse, little or no dendritic branching occurred. On the other hand, if little or no axonal branching was observed, dendritic sprouting was profuse. Intermediate situations, in which a modest amount of branching occurred in both compartments, were also observed.

We propose that the MGI reacts to axotomy by synthesizing membrane and inserting it into sprouts at a constant rate. The distribution of this sprouting membrane, however, is apportioned differentially between the axonal and dendritic compartments. If the proximal axon stump remains relatively intact, sprouting will occur preferentially at the proximal axonal end. At the other extreme, where the axon has died back completely, the dendritic compartment is available to accept newly synthesized membrane, and hence the profuse supernumerary dendritic outgrowths observed in such situations. In the intermediate case, where partial axon die-back occurs, only a portion of the total sprouting membrane can be inserted at the proximal axonal stump. The balance of newly synthesized membrane is inserted into the dendrite, thereby conserving the total area of sprouting membrane.

Finally, the nature of the growing neurites induced in three distinct neuronal compartments, the soma, dendrites, and axon, appears structurally similar. This suggests that the properties of the newly synthesized membrane, destined for insertion into the three cellular compartments, is initially similar. Only later, after formation of the new structure has progressed, might the attributes specific to the newly formed element then differentiate, as is known to occur during development (Spitzer, 1979). Therefore, what is affected by altering the site of the lesion may be the intracellular distribution system. It is this system of internal transport, encompassing elements of the cytoskeleton (Lasek, 1981), that is most likely involved with determining where the new membrane is inserted in the regenerating neuron remnant. It is the effect of close and distant lesions on the properties of the cell body, including cytoskeletal elements of the internal transport system, that is considered in the following paper (Roederer and Cohen, 1983).

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