REGENERATION OF AN IDENTIFIED CENTRAL NEURON IN THE CRICKET

II. Electrical and Morphological Responses of the Soma¹

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Abstract

The cell body of the medial giant interneuron (MGI) in the cricket normally does not spike in response to injected depolarizing currents. When axotomized 1 mm or more from the cell body (distant axotomy), the membrane properties of the soma remain unchanged. However, after axotomy close to the cell body (200 to 500 μ m), the soma membrane becomes capable of generating action potentials by 6 hr after lesion. These regenerative spikes are 1 to 1.5 msec in duration and may reach 100 mV in peak amplitude. Ion substitutions indicate that these action potentials are primarily sodium dependent. A calcium-dependent component of soma membrane excitability that is normally present appears to be unaffected by axotomy. By 48 hr, the close axotomized MGI somata have lost the ability to generate action potentials and the membrane electrical properties return to normal.

By 2 days after axotomy close to the soma, large, membrane-bound, electron-lucent vacuoles appear in the cytoplasm of the MGI cell body. Such vacuoles then disappear from axotomized MGI somata by 10 days. In addition, numerous arrays of densely packed, darkly staining microtubules are observed in the cell body, especially concentrated near the initial neurite. Neither of these specialized structures is observed in control, intact MGI somata.

We propose that close axotomy disrupts the mechanisms which regulate the stability of the fully mature, differentiated neuron. The characteristic morphological and physiological stability of the MGI is lost: the dendritic arborization has been shown previously to be altered by extensive new outgrowth (Roederer, E., and M. J. Cohen (1983) *J. Neurosci.* 3: 1835-1847); there is a transient increase in soma membrane excitability, and new cytoplasmic organelles are induced.

When the axon of a neuron is cut, its cell body undergoes a series of well characterized metabolic changes loosely included in the concept "axon reaction" (reviewed by Cragg, 1970; Lieberman, 1974; Grafstein, 1975; Watson, 1976; Sunderland, 1978). The distance of the site of axon transection from the cell body is thought to alter the intensity, onset, and duration of this response (e.g., Yin et al., 1981). Lesions closer to the cell body usually cause an earlier onset and a more intense, briefer response. Several hypotheses have been put forward to explain the timing and gradation of this response as a

function of the distance of lesion from the soma (Cragg, 1970). The underlying concept is taken to be that the soma response itself remains qualitatively the same for all sites of axonal transections, and that varying the distance of lesion from the soma only quantitatively enhances or depresses some parameters of this response. However, we observed in this and in a previous study (Roederer and Cohen, 1983) that, for an identified interneuron in the central nervous system of the cricket, the medial giant interneuron (MGI), the nature of the response changes when the axon is injured close to the cell body.

Previously, we showed that the following responses are induced by *distant* axotomy of the MGI (Roederer and Cohen, 1983). (i) The axon generates neurites which, after a crush, cross the lesion and continue growing, whereas, after a cut, they generally remain in the vicinity of the proximal stump to form a "neuroma." (ii) The characteristic dendritic morphology of the MGI remains stable at all times, up to 72 days after such lesions.

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For *close* lesion, we demonstrated that: (i) the proximal axonal stump often dies back several hundred micrometers from the site of lesion and sometimes produces only a few, underdeveloped sprouts; (ii) however, the dendrites of MGIs lesioned close to the cell body generate considerable supernumerary sprouts that grow throughout the neuropil and may enter nerves never occupied by the normal MGI arborization.

In this report we further show that *distant* axotomy of the MGI does not alter the electrophysiological properties of the cell body membrane at any time post-transection. On the other hand, *close* axotomy of the MGI, at 200 μ m from the terminal ganglion, induces the following responses which are different from those evoked by a more distant transection. (i) The cell body of the MGI, which normally does not spike, begins to show overshooting, sodium-dependent action potentials as early as 6 hr post-axotomy, and these spikes then disappear at about 48 hr. (ii) Large, clear, membrane-bound vacuoles appear in the cytoplasm 2 days postoperative and disappear after about day 10. In addition, numerous ordered, parallel arrays of short microtubules can be seen in the cytoplasm, concentrated especially near the initial neurite.

Our observations, taken together from this and a previous paper (Roederer and Cohen, 1983), strongly indicate that the precise nature of the structural and physiological changes induced in the MGI by axotomy is determined by the distance of the lesion from the soma. In particular, we propose that a close lesion can disrupt the mechanisms which generate and maintain the genetically determined physiological and morphological properties of a fully differentiated neuron.

Materials and Methods

Operations and electrophysiology

One-day-old adult, male crickets, *Acheta domesticus*, were raised, operated, and prepared for electrophysiology as previously described (Roederer and Cohen, 1983).

Ion substitutions. The following solutions were employed to investigate the ionic dependence of induced soma action potentials: normal saline (140 mM NaCl, 10 mM KCl, 2 mM CaCl_2 , 6 mM Na_2HPO_4 , 4 mM NaH_2PO_4) (Usherwood and Grundfest, 1965); *O*-sodium saline, designated *O*- Na^+ (140 mM choline chloride, 10 mM KCl, 2 mM CaCl_2 , 10 mM Tris); saline containing barium, designated Ba^{2+} (90 mM NaCl, 10 mM KCl, 10 mM Tris, 50 mM BaCl_2); and saline containing barium and cobalt, designated $\text{Ba}^{2+}, \text{Co}^{2+}$ (90 mM NaCl, 10 mM KCl, 10 mM Tris, 40 mM BaCl_2 , 10 mM CoCl_2). All solutions were buffered to pH 6.85.

The terminal ganglion of the cricket is surrounded by a thick, tough sheath. This was probably responsible for the long immersion times frequently required in the experimental solutions (30 min to 1 hr) before effects could be detected electrophysiologically from neurons in the ganglion.

Morphology

Ganglia from experimental animals were fixed using a protocol previously developed for electron microscopy of insect nervous tissue (Wood et al., 1977). Tissue was sectioned at 3 μ m, stained with 0.5% toluidine blue in

1% borax, and subsequently examined and photographed under phase contrast optics on a Zeiss Universal microscope.

For examination of the MGI soma in the electron microscope, the ganglion was thick-sectioned serially until the cell body was reached, at which point thin sections were cut from the block face. Such sections were placed on single-slot grids, stained with uranyl acetate and lead citrate, then examined and photographed on a Phillips 300 transmission electron microscope.

Results

Stability of normal soma membrane properties following distant axotomy

The normal MGI soma does not spike (Murphey, 1973) and responds passively to hyperpolarizing current pulses with hyperpolarizing membrane voltage deflections linearly proportional to the magnitude of the injected current. As the magnitude of depolarizing current pulse is increased (Fig. 1, *a* to *c*), the soma membrane responds with increasing depolarizing deflections only until a steady-state voltage of maximum conductance ($V_{g_{\max}}$) is reached. Beyond this value, increasing the injected current does not elicit higher, steady-state membrane voltage deflections. This can be represented on a standard I-V plot of injected current versus resultant voltage deflection from resting membrane potential, as shown in Figure 2. The plateau voltage is indicative of a rectifying membrane current activated by depolarization (Connor and Stevens, 1971). Regenerative action potentials cannot be elicited from normal MGI somata, as is also the case in normal insect motoneurons (Pitman et al., 1972; Goodman and Heitler, 1979) and many intact crayfish nerve cell bodies (Kuwada and Wine, 1981).

Following distant cuts of the MGI axon, the cell body membrane properties remained unchanged at all times after injury. I-V plots drawn for distantly axotomized cells were not different from those of normal, intact cell bodies (Fig. 2). The resting membrane potential, the input resistance (R_i) to hyperpolarizing current pulses, and the voltage of maximum conductance ($V_{g_{\max}}$) remained within the normal range for all MGIs cut distant from the terminal ganglion, i.e., at t_3 to t_5 (as in Fig. 1, Roederer and Cohen, 1983).

Transient spikes induced in the soma following close axotomy

Description of the phenomenon. In order to study the response of the MGI cell body to proximal cuts, one of the connectives was severed at approximately 200 μ m from the terminal ganglion (t_1 in Fig. 1, Roederer and Cohen, 1983). In a preparation at 24 hr after this type of injury, the MGI soma demonstrated regenerative action potentials measuring 1.5 msec in duration and 90 mV in amplitude, well overshooting the zero membrane potential of 61 mV (Fig. 1, *d* to *f*).

By 2 days after close axotomy, the MGI soma no longer spiked, but displayed normal membrane properties indistinguishable from those of intact neurons. Figures 3 and 4 show intracellular records taken from seven MGIs, following close cuts, representing a range of post-axotomy times from 10 min to 49 hr. Ten minutes after close

Figure 1. Membrane voltage responses (upper trace) of the MGI soma to increasing levels of injected current (lower trace). *a* to *c*, Note the lack of induced action potentials from a normal soma. *d* to *f*, Records from a cell body 24 hr after lesion at t_1 (see Fig. 1 Roederer and Cohen, 1983). The fast action potentials evoked in the axotomized neuron measure approximately 90 mV in amplitude. Note the difference in calibration in the two cases.

cut (Fig. 3, *a* to *c*) the membrane properties of the MGI soma remain indistinguishable from normal. This means that the site of axon transection is sufficiently distant electrotonically from the soma that the injury itself does not cause immediate alterations in the resting membrane potential or other soma properties.

About 4 hr post-axotomy, a 20-mV broad peak appears at the onset of the depolarizing voltage deflection (Fig. 3, *d* to *f*). By 8 hr post-axotomy, an excitable component is seen at increasing depolarizing currents (Fig. 3, *g* to *i*). Note the increased amplitude of the response elicited at 8 hr compared with only a flat plateau response at 10 min, which is similar to a normal MGI response.

Figure 3, *j* to *l*, from a preparation 19 hr after axotomy at location t_1 , shows fully developed, overshooting action potentials in response to depolarizing current pulses.

Figure 2. Plot of injected current (nA) versus resulting membrane deflection from the resting potential (mV) for three MGI somata: a representative control, and two cell bodies 18 and 54 days after axotomy at t_3 . The curves are linear in the hyperpolarizing direction and level off in the depolarizing quadrant. For each curve, the input resistance R_i is calculated as the slope of the curve in the hyperpolarizing quadrant. The voltage of maximum conductance, V_{gmax} is defined as the plateau voltage value in the depolarizing quadrant.

Increasing the amplitude of the applied current increases the frequency of spiking. This phenomenon is still apparent at 31 hr post-axotomy (Fig. 4, *a* to *c*). However, by 48 hr the amplitude of the action potential has fallen substantially and the threshold has risen concomitantly (Fig. 4, *d* to *f*). In fact, by 48 hr some preparations show either much diminished responses to depolarizing current pulses or no action potentials at all (Fig. 4, *g* to *i*).

The transient time course of the induced action potential can be seen in a plot of its peak amplitude as a function of postoperative time (Fig. 5). All 23 cells examined between 9 and 36 hr post-axotomy displayed action potentials measuring at least 55 mV in amplitude. At times less than 9 hr or greater than 36 hr, either smaller amplitudes or no action potentials at all were observed.

With the exception of generating action potentials, other membrane properties of the 29 spiking somata remained unchanged. Table I shows that the resting membrane potential and input resistance to hyperpolarizing current pulses of this group of spiking cells were within the range of values observed in intact, nonspiking somata.

The possibility still remained that the appearance of the transient spike in the MGI following close cut of the connectives results from systemic influences arising from injury. To test for such a systemic effect, we examined 7 MGIs contralateral to such close cuts ranging over a period from 8 to 36 hr after injury. No spikes were induced in this control group, and the electrophysiological properties were indistinguishable from those of normal, intact MGIs or from interneurons axotomized at a

Figure 3. Intracellular records (upper trace) from axotomized MGI somata at various times after cutting a connective at site t_1 . Within each time period the level of injected current (lower trace) increases from left to right. At 4 hr after lesion the start of an induced transient response to injected current is seen (f).

distance of 2 mm or more from the terminal ganglion (Table I). Hence, the induction of the excitable component following a close cut is attributable to axotomy itself and not to systemic influences of injury.

The electrical excitability induced by close axotomy disappears at about 48 hr and does not return. Twenty MGIs were examined at 3 to 12 days following close axotomy. The electrical properties of these somata were not substantially different from those of normal cells (Table I).

The nature of the spike induced by close axotomy. From a series of ion substitution experiments carried out on spiking MGI cell bodies, it became evident that the action potential induced by axotomy is sodium dependent. With the microelectrode positioned intracellularly in such a soma, the saline bathing the preparation was exchanged for 0-sodium saline in which choline ions replaced so-

Figure 4. Intracellular records (upper trace) from MGI somata following cuts at t_1 at various times during the 2nd day after lesion. Increasing values of injected current (lower trace) from left to right. Note the disappearance of the evoked action potentials by 49 hr after axotomy.

Figure 5. Scatter plot of soma excitability induced by close axotomy at location t_1 . The measured amplitudes of evoked action potentials for each of 56 axotomized somata are plotted at various times following close transection. Zero values indicate the absence of regenerative spikes but otherwise completely normal membrane properties.

dium. The outcome of such an experiment is shown in Figure 6, carried out on a spiking MGI soma 6 hr after close axotomy. Following substitution of sodium by choline, there was an initial fall in the amplitude of the action potential and an increase in the threshold (Fig. 6b). Eventually, the action potential disappeared altogether (Fig. 6c). After replacement of choline chloride saline by normal saline, excitability gradually returned

TABLE I

Summary of membrane properties in control and axotomized MGI somata

The data from all MGI somata axotomized at different distances from the terminal ganglion are divided into two groups: those MGIs examined at times less than 2 days after lesion, and those examined at times longer than 2 days. The averaged values for the resting membrane potential, the input resistance to hyperpolarizing current pulses (R_i), and the membrane voltage at maximum conductance (V_{gmax}) are given for each group. Control preparations are records obtained from the intact MGI somata contralateral to neurons axotomized at t_1 . Note that induced spikes are seen only for lesions within 400 to 500 μ m of the ganglion and within 2 days after axotomy. All other parameters are not significantly different from the normal range.

Distance of Lesion	Time (days)	N	Membrane Potential (mV)	R_i (megohms)	V_{gmax} (mV)
Normal		37	61 ± 4	6.4 ± 1.4	6.6 ± 2.1
200 μ m	≤ 2	29	61 ± 4	6.3 ± 1.3	spiking
200 μ m	> 2	20	56 ± 7	6.3 ± 2.3	6.9 ± 3.2
Control for 200 μ m	≤ 2	7	59 ± 5	6.1 ± 1.2	7.0 ± 3.1
Control for 200 μ m	> 2	5	62 ± 3	6.8 ± 0.9	8.2 ± 4.0
400–500 μ m	≤ 2	11	61 ± 6	6.6 ± 1.3	some spiking
400–500 μ m	> 2	6	56 ± 5	5.8 ± 1.4	7.3 ± 4.1
≥ 1900 μ m	≤ 2	8	62 ± 3	6.4 ± 1.1	9.1 ± 3.5
≥ 1900 μ m	> 2	13	58 ± 6	6.2 ± 1.1	9.9 ± 3.9

(Fig. 6d), although the initial amplitude of the action potential was often not fully restored (Fig. 6e).

In eight spiking MGI somata examined at 6 to 29 hr post-axotomy, choline chloride abolished the induced action potential. Since the other ions in choline chloride saline, such as calcium, potassium, and chloride, remained at their normal concentrations, it is clear that the action potential induced by axotomy shows strong, if not exclusive, sodium dependence.

In an attempt to manipulate the ionic environment indirectly to test for possible calcium dependence of the action potential, barium was found to be a reliable cation substitute for calcium. From analogous systems among vertebrates and invertebrates, it is known that barium has several effects on the electrophysiology of cells (Hagiwara et al., 1974; Schwandt and Crill, 1980; reviewed in Hagiwara, 1980): (i) barium partially blocks the voltage-dependent potassium conductance; (ii) as a divalent cation, barium is more effective than calcium in carrying charge through the voltage-dependent calcium channel; (iii) barium partially blocks the calcium-dependent potassium conductance.

The intact MGI soma, similar to many normal vertebrate and invertebrate somata, produces overshooting action potentials when barium is present in the saline (Fig. 7, a to e). Such barium action potentials are probably caused by an underlying, voltage-dependent calcium conductance revealed by two effects of barium (Hagiwara et al., 1974): (i) partial or total block of rectifying current, and (ii) substitution for calcium as the current-carrying ion flowing into the cell through the calcium channel when it is activated by membrane depolarization.

Figure 7, a to c, shows such representative barium action potentials from a normal MGI soma bathed in saline containing barium chloride. These responses can be elicited by long depolarizing current pulses (Fig. 7b)

Figure 6. Intracellular records (upper trace) obtained from an MGI soma whose axon was cut at t_1 6 hr previously, and which displayed action potentials to depolarizing current pulses (lower trace) a, Response in normal saline to injected current. b, After 5 min in bath with all sodium replaced by choline. Note the increase in threshold and the decrease in spike amplitude. c, Same as b, after 30 min; note absence of spikes. d, Five minutes after returning to normal saline, note return of spike. e, Same as d, after 30 min. Repetitive spiking and larger action potential amplitudes are observed, although still below the initial value in a.

or they can also be triggered by a short depolarizing current pulse at threshold (Fig. 7c). Five normal MGIs bathed in barium chloride showed long-lasting action potentials similar to those shown in Figure 7, b and c. It thus became apparent that the normal MGI soma membrane possesses a calcium conductance system, but one that is not electrophysiologically evident in normal saline and using depolarizing currents of the order of 10 nA.

The presence of barium action potentials was tested in axotomized cells as well. In all 11 MGIs tested following close axotomy, such a response could be evoked. This included five spiking somata following a close cut at t_1 , two close-cut somata at times greater than 2 days and thus no longer spiking, and four distant cut MGIs which never had spiking cell bodies. It was clear that, following axotomy, barium action potentials were not lost by the MGI.

When barium action potentials were evoked in spiking somata (Fig. 7, d to h), the prolonged duration of the barium response was seen to sum with the fast sodium spikes. As the barium response first develops (Fig. 8b), repetitive, fast spikes are observed on the plateau of the incipient, underlying barium action potential. After the

Figure 7. *a*, Intracellular (upper trace) response to injected current (lower trace) from a normal MGI soma in normal saline. *b*, Addition of saline with barium to the bath elicits a long-lasting barium action potential. *c*, Barium action potential can also be evoked by short-duration, intracellular current pulse (vertical calibration for current in this record, 50 nA). *d*, Record taken for an MGI soma 22 hr following axotomy at t_1 , showing induced spikes in normal saline. *e*, Replacement of sodium by choline abolishes the spike. *f*, In normal saline the spike returns. *g* and *h*, Addition of barium to the saline reveals a two-component action potential: a fast sodium spike at the onset, followed by a long-duration barium potential. Notice the brief-duration component is lacking from the barium potentials seen in the normal MGI soma, *b* and *c*.

barium potential has developed fully, fast spiking is no longer observed during the interval of the peak barium response, but fast spikes still occur at the onset and during inactivation of the barium response (Fig. 8, *c* and *d*). Application of cobalt to this barium saline eliminated most of the barium action potential, presumably by blocking the calcium channels through which the barium is flowing (Hagiwara et al., 1974; Schwandt and Crill, 1980); this once again permitted repetitive sodium spikes (Fig. 8*e*). It is significant that cobalt inhibited the barium potential but left the sodium action potentials intact. The reduced amplitude of the sodium spikes (cf. Fig. 8, *a* and *e*) can be attributed to the reduction of sodium concentration from 140 mM to 90 mM to compensate for the presence of 50 mM barium chloride. This would reduce the E_{Na} , as calculated from the Nernst equation,

Figure 8. Intracellular response (upper trace) to injected current (lower trace) from an MGI soma 19 hr after axotomy at t_1 . *a*, Action potential as seen in normal saline. *b*, After 5 min incubation in barium saline, the MGI displays a small, prolonged barium action potential crowned with fast, diminished sodium spikes. *c*, After 20 min incubation the barium action potential has become larger in amplitude, and the only fast spiking component is seen at the onset and at the end of the barium response. *d*, After 35 min incubation. *e*, Addition of cobalt to the barium-containing saline markedly reduced the barium action potential while again permitting repetitive sodium spiking.

and thus the maximum amplitude achievable by a primarily sodium-dependent action potential.

In summary, these experiments indicate that the action potential induced by close axotomy is sodium dependent, and *independent* of an underlying calcium conductance system whose parameters are not significantly affected.

Effect of increasing the distance of the lesion site from the soma. Different responses were obtained by cutting the MGI axon at either 200 μ m from the terminal ganglion or at distances of 2000 μ m or greater. Thus, the question arose whether the induction of the soma action potential was an all-or-none phenomenon dependent on some threshold distance of axotomy from the ganglion. An intermediate location for transection was explored to test for the possibility of intermediate responses. A series of 17 MGI somata were sampled following axon cuts at t_2 , 400 to 500 μ m from the terminal ganglion (Fig. 1,

Figure 9. Intracellular records (upper trace) from MGI somata axotomized at t_2 (400 to 500 μm from the terminal ganglion), at 21 (a to c), 30 (d to f), and 48 (g to i) hr after lesions. Left-to-right progression of increasing injected current amplitudes (lower trace). Note the reduced amplitude of the action potentials induced by this more distant lesion (cf. Figs. 3 and 4).

Roederer and Cohen, 1983). Nine of these MGIs were tested at times 8 to 48 hr post-axotomy, a time corresponding to the period in which the majority of close cut MGIs spiked (Fig. 5).

It became apparent (Fig. 9) that "intermediate" responses could be elicited during this time period. These MGIs showed a diminished regenerative response with a higher threshold compared to close cut MGIs (Fig. 9, a to f). By 48 hr even this diminished excitability could no longer be evoked (Fig. 9, g to i). In eight MGIs tested at 3 to 12 days following a cut at t_2 , no spike could be evoked. This corresponded in time to the period when once-spiking MGI somata cut at t_1 had also become inexcitable (Fig. 5). Other soma membrane properties of these MGIs, such as the resting membrane potential and the input resistance to hyperpolarizing current pulses, remained within the normal range at all times (Table I).

Choline chloride substitution for sodium in the saline revealed that the excitable component induced by intermediate lesions at t_2 also showed sodium dependence. The small action potentials evoked by depolarizing current pulses were lost in choline chloride but returned when sodium was replaced in the saline.

Vacuoles and microtubular arrays induced in the soma by close axotomy

In addition to these electrophysiological events, a search was undertaken for morphological changes induced in the soma by close cut of the axon. Control and close cut MGIs were sectioned at various times following transection at location t_1 (200 μm from the terminal

ganglion). Two days following such transections, large, membrane-bound vacuoles began to appear in the cytoplasm of injured cell bodies. Examples of such vacuoles induced by axotomy are shown in the light micrographs of Figure 10, b to d. They are well developed by day 2 and persist for at least 1 week. By days 9 and 10 they can be detected only occasionally, and at later times, up to 24 days, no such structures are seen again. These vacuoles were found in 11 of 14 axotomized MGIs in the period 2 to 6 days after close axotomy. Such cytoplasmic structures were never seen on the control-side homologues in the same preparation, whose axon was left intact by unilateral ventral nerve cord transection. The vacuoles tend to be rounded, their contents do not stain with toluidine blue, nor are they electron dense (Fig. 11, a and b). Although they are found most often in the peripheral cytoplasm of the axotomized soma, they can also be seen toward the middle of the cell surrounded by normal cell organelles.

Close cut axotomized MGI somata also demonstrated numerous arrays of densely packed microtubule-like structures (Fig. 12). They were especially concentrated in the area where the initial neurite emerges from the cell body. These microtubular aggregates were found in five of seven preparations examined ultrastructurally 4 to 6 days after lesion of the axon. In a single section through an MGI cell body, 50 to 90 of these arrays could be seen. An individual aggregate is composed of single tubular subunits 9 to 15 nm in diameter and 100 to 300 nm long. These are more electron dense than the normal, isolated, longer microtubules. They also appear to have a distinct halo of electron-dense material around their cylindrical core. Up to 250 tubular units may be found in a single aggregate. Isolated microtubules coursing through the cytoplasm are a normal ultrastructural component of the MGI soma. The aggregates induced in axotomized cells occurred in addition to a seemingly normal complement of isolated microtubules. Control MGI somata on the contralateral side displayed the normal complement of isolated microtubules in the cytoplasm and no evidence of the densely packed aggregates.

Discussion

Change in soma membrane excitability induced by close axotomy. In many neurons axotomy not only causes profound metabolic and structural changes in the cell body (Cragg, 1970; Lieberman, 1974; Grafstein, 1975; Watson, 1976; Sunderland, 1978), but may also induce alterations in the electrical properties of the cell membrane (Heyer and Llinas, 1977; Gustafsson, 1979). Among invertebrates, cell bodies which normally do not spike show regenerative, overshooting action potentials about 4 days after axotomy (Pitman et al., 1972; Goodman and Heitler, 1979; Kuwada and Wine, 1981).

We have shown that the electrophysiological response of the MGI soma to axotomy can vary. These differences in response correlate directly with the distance of the lesion from the terminal ganglion. Like the cell body of many invertebrate neurons, the membrane of the MGI soma does not normally generate action potentials (Murphy, 1973). Following a distant cut, the electrophysiological properties of the MGI soma remain unchanged.

Figure 10. Representative sections from different MGI somata stained with toluidine blue and viewed in phase contrast: *a*, normal MGI; *b*, 2 days; *c*, 4 days; and *d*, 6 days after close axotomy at t_1 . Arrows indicate the presence of large, pale vacuoles in the cytoplasm of the axotomized MGIs. Thin sections for electron microscopy were obtained from the preparation in *d* and are shown in Figure 11.

However, soon after close axotomy, the MGI cell body will produce sodium-dependent action potentials, similar to those induced in the invertebrate preparations mentioned above. In the cricket MGI, the induced spikes are gone from the soma membrane by 2 days after axotomy.

This phenomenon raises the question: What is the mechanism whereby sodium-dependent spikes transiently appear in close cut MGI somata? We consider three testable possibilities. (i) Axotomy close to the soma initiates or accelerates the synthesis of voltage-gated, sodium channels; then some of these channels are inserted into the soma membrane, which is normally free of sodium channels. (ii) Alternatively, no change in the synthesis of the channel proteins occurs. Close axotomy simply removes most of the potential target for the insertion of such proteins, leaving only dendrites and soma membrane available for addition of new sodium channels. (iii) Finally, voltage-gated sodium channels are always present in the soma membrane but are normally inactivated; axotomy transiently "uncovers" such channels and permits sodium spikes in the soma membrane.

Sodium channels can indeed be induced transiently in *Xenopus* oocytes by simply depolarizing the membrane to +50 mV (Baud et al., 1982). However, in the MGI, no differences in the resting membrane potential were observed between control and axotomized somata at any time following injury. Thus, an activation of sodium channels that is dependent on a sustained depolarization, as in the oocyte, is unlikely in the MGI.

The first two possibilities seem more likely because they can also account for the eventual loss of excitability from the MGI soma. Close axotomy does remove a significant proportion of the total cell membrane. Protein synthesis has been shown to be necessary for the induction of excitability in axotomized crayfish neurons (Kuwada, 1981). If we assume that synthesis of sodium channels, destined for axonal membrane, either continues or is accelerated by axotomy, then new channels may accumulate in the soma cytoplasm because their usual site of insertion, the axon, has been removed. This could result in their temporary insertion into the closest available target area, i.e., the soma membrane, and result in the appearance of the sodium-dependent action potentials in the soma observed by 6 hr after close axotomy.

Extensive sprouting from the dendrites of the MGI is seen by 2 days after such close axotomy (Roederer and Cohen, 1983). At this time, newly added membrane in the form of sprouts could provide an alternative target for insertion of sodium channels and thereby spare the soma membrane. Given a steady turnover of channel proteins, this would account for the loss of sodium-dependent excitability from the soma by 48 hr after injury. With distant axotomy, the remaining axonal stump provides a sufficiently large target area for new sodium channels that they need not accumulate in the soma membrane. Thus there is no change at any time in soma excitability following the more distant axonal injuries.

How axotomy influences the soma. The present work and the previous paper (Roederer and Cohen, 1983) show that the response of the soma and the dendrites to axotomy depends on the site of axon transection. Not

Figure 11. *a*, Electron micrograph of the MGI seen in Figure 10*d* showing several large, membrane-bound vacuoles (V) in the cytoplasm, the largest of which measures approximately 5 μ m in diameter. The content of the vacuoles is electron lucent. *b*, Higher magnification of an area near the large vacuole in *a*, showing the membrane around the vacuole and the nearby aggregations of microtubules (*mt*).

only the magnitude of the response, but its very nature is determined by the distance of the lesion from the soma. Lieberman (1974) has suggested that the variable response of the soma to different locations of the axon lesion may be due to an inhibitory trophic influence exerted in a retrograde direction by postsynaptic targets via axon collaterals. However, it is unlikely that a trophic effect originating from postsynaptic structures determines the response of the cricket MGI to axotomy. This interneuron does not have any collaterals within the interganglionic connectives between transection sites t_1 and t_3 (Fig. 1, Roederer and Cohen, 1983). Yet a close cut at t_1 leads to an entirely different response from the parent MGI soma than does a more distant cut at t_3 , even though both lesions are within the same intersegmental connective. Therefore, this difference in response cannot be ascribed to a differential removal of an inhibitory influence due to the detachment of varying numbers of postsynaptic elements.

It thus appears that the factors responsible for the differences between close and distant axotomies are critically dependent upon the actual distance of the lesion from the soma. We see the distal lesion as having little effect on the nature of anabolic activity except perhaps to change its rate, as indicated in other systems by increased levels of RNA production and altered rates of protein synthesis (Grafstein, 1975; Grafstein and McQuarrie, 1978). The distinctive form of a specific, differentiated neuron is clearly retained during this type of response to a distant injury.

However, with axotomy *close* to the soma, the basic structural and physiological characteristics of the differentiated neuron are profoundly altered. The control systems that maintain the distinctive, polarized structure of the mature neuron and the precise distribution of membrane properties are disrupted. In the extreme case, the proximal axonal stump disappears and elongating, supernumerary neurites emerge from the dendritic arborization at the same time that the soma membrane transiently produces overshooting action potentials.

In addition, the close cut is also correlated with the appearance of large, membrane-bound vacuoles in the soma cytoplasm similar to those induced by axotomy in the central nervous system of higher vertebrates (Barron et al., 1971, 1973). Close axotomy also can alter elements of the cytoskeleton by inducing aggregates of short, electron-dense microtubules which are particularly concentrated in the region where the initial neurite originates from the soma. Studies on the goldfish retina have shown that axotomy can specifically affect microtubule metabolism at the level of the genome by accelerating the synthesis of the principal microtubule component, tubulin (Heacock and Agranoff, 1976; Burrell et al., 1979). The distinctive aggregates of short, dense microtubules induced by axotomy in our study may play a particular role in dendritic sprouting. Bernhardt and Matus (1982) report an increase in a high molecular weight microtubule-associated protein related specifically to the growth of dendrites in embryonic Purkinje cells. It is possible that the distinctive microtubules induced by close axo-

Figure 12. Electron micrographs showing densely packed aggregates of microtubules induced in MGI somata by axotomy. a, Six days after axotomy. Two aggregates of short, dense, regularly arrayed microtubules are apparent. Such short microtubules can also be seen in disarray (arrow). b and c, Four days after axotomy. Higher magnification of the induced microtubular aggregates seen in cross-section (b) and in longitudinal section (c). Such aggregates are commonly seen near glial trophospongia (tr).

tomy in the cricket also are associated with a product required by the induced dendritic outgrowth.

We propose that the effects on neuron form and ultrastructure induced by close axotomy are due in part to an influx of cations, in particular calcium, as has been suggested by Jaffe (1980) for other systems undergoing developmental change. Sodium and calcium comprise the major ionic components of the massive injury current that enters the cut face of the transected lamprey spinal cord (Borgens et al., 1980). Such an inward current of injury was also observed entering the cut interganglionic connectives in the cricket (E. Roederer and M. J. Cohen, personal observations). Similarly, Meiri and co-workers (1981) report an increase in cation conductances (potassium, sodium, and calcium) that persists for several days after injury at the cut end of giant interneuron axons in the cockroach. In addition, they observed calcium-dependent action potentials at the growing terminal of the proximal axon stump. The large vacuoles found in the MGI soma following close axotomy are similar to those seen in another neuron after a direct injection of calcium ions into its cell body (Nicaise and Meech, 1980). We propose that the vacuoles induced by close axotomy in the MGI are caused by an increased concentration of calcium in the soma cytoplasm resulting from an influx into the axon at the site of lesion.

The concentration of calcium is also known to play a fundamental role in controlling the assembly and breakdown of fibrous proteins in the cytoskeleton, including microtubules (Wilson et al., 1970; Borisy and Olmsted, 1972; Weisenberg, 1972; Kirschner and Williams, 1974) and neurofilaments (Gilbert et al., 1975; Pant et al., 1979). The alterations in microtubular structure indicated by the appearance of highly ordered aggregates of short, dense microtubules could also be brought about by an increase of intracellular calcium levels due to the influx at the site of injury. Microtubules are involved with the transport of materials and the assignment of such materials to particular destinations within the cell (Grafstein and Forman, 1980; Whitnall and Grafstein, 1982). Thus, these changes in microtubule organization may reflect a shift in the distribution of newly synthesized membrane. This redistribution of new membrane components may cause the uncontrolled neuritic growth from the dendritic tree and the appearance of action potentials on the soma membrane, rather than permitting an orderly regeneration of the severed axon.

In a cell such as a neuron, where highly differentiated structure is intimately related to its functional specialization, form and function remain relatively stable once developmental differentiation has occurred. With a more distant axonal lesion, the concentration of inflowing cations at the site of injury may not reach levels in the soma sufficient to alter the basic regulatory mechanisms located there. Thus the structure and function of the distantly injured neuron remnant is retained while a controlled regenerative process takes place at the end of the proximal axonal stump. However, with a lesion close to the soma, the concentration of cations in the soma may increase sufficiently to overwhelm the known intracellular buffering systems (Baker, 1970). This in turn could result in the major changes of neuron form and

function reported here and in the previous paper (Roederer and Cohen, 1983).

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