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Microtubule destabilization and neurofilament phosphorylation precede dendritic sprouting after close axotomy of lamprey central neurons

(dendritic identity/neuronal polarity/axonal regeneration/*Petromyzon marinus*)

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ABSTRACT **Axotomy of giant lamprey (*Petromyzon marinus*) central neurons (anterior bulbar cells) close to their somata results in ectopic axon-like sprouting from the dendritic tips. Such sprouts first appear as swellings at the tips of a small subset of dendrites 2–3 weeks after "close" axotomy. We report here that immunocytochemical examination of these swellings reveals a structure and composition that differs from that of conventional growth cones; incipient sprouts contain many highly phosphorylated neurofilaments (NFs), little tubulin, and virtually no stable (acetylated) microtubules (MTs). The dendrites of anterior bulbar cells after close axotomy also show pronounced changes in NF protein and tubulin staining patterns prior to the emergence of sprouts from the dendrites. The amount of tyrosinated tubulin increases greatly; this rise is tightly coupled to the appearance of highly phosphorylated NFs and the loss of nonphosphorylated NFs in the dendrites. Acetylated tubulin is generally reduced after close axotomy and is selectively lost from dendrites that gave rise to sprouts. These changes indicate that an invasion of the dendrites by phosphorylated NFs may be linked to the destabilization of dendritic MTs, and in some dendrites this may lead to a marked loss of stable MTs, which is correlated with the emergence of NF-filled sprouts from the dendritic tips.**

Axotomy leads to a variety of changes in the affected neuron [i.e., chromatolysis, an increase in phosphorylated neurofilaments, changes in the electrical properties of the plasma membrane, dendritic retraction, etc. (1–8)], some of which are probably important preliminaries to axonal regeneration. Most cellular consequences of axotomy are more marked after axotomy close to the soma than after more distant lesions (1). "Close" axotomy has also been shown in several systems to cause a fundamental cellular change that does not occur at all after distant axotomy—i.e., a loss of the ability to regenerate the axon specifically from the axon stump, resulting in axonal regeneration from the dendritic tips as well as (or instead of) from the axon stump in a number of systems (6, 9–11). This loss of normal cellular polarity allows one to ask what the mechanisms are that control the site of axonal regeneration within the cell and also permits one to study the earliest events of axonal regeneration in a different context. Because axonal regeneration by definition occurs from an injured process (the axon stump), distinguishing early regenerative events from degeneration induced by local injury is normally impossible. This difficulty can be circumvented by studying ectopic axonal regeneration from uninjured dendrites, which permits one to more directly compare the early events of axonal regeneration with axonal development.

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We have chosen a set of identified central neurons [anterior bulbar cells (ABCs)] in the lamprey (*Petromyzon marinus*), a primitive vertebrate, to study the events leading up to axon-like sprouting from the dendrites at early times after close axotomy. Such sprouting has already been investigated in some detail in lamprey ABCs (8, 9, 12–14), where dendritic sprouts have been observed to emerge from the dendritic tips by 2–3 weeks after close axotomy and to grow steadily for many weeks along typically axonal trajectories. Such sprouts elongate at mean rates of up to 100 μm per day and often reach lengths of several millimeters. Dendritic sprouting is accompanied by major cytoskeletal rearrangements in the dendrites of the affected cell by 8–10 weeks after the onset of sprouting (14). The cytoskeleton of the sprouts consists almost entirely of neurofilaments (NFs), with very few microtubules (MTs). This composition is similar to that of intact and regenerating ABC axons, but it is unlike that of either intact ABC dendrites (14) or developing axons and their growth cones in other systems, which have few if any NFs (15, 16). This raises the question of whether these sprouts had ceased growing by the time they were examined or if they elongate in a manner unlike axons in developing systems or *in vitro*. The goals of the present study have been (*i*) to identify those consequences of close axotomy that may be important in the loss of normal polarity by studying the cytoskeletal changes that occur in ABCs before and during the onset of axon-like sprouting from the dendrites and (*ii*) to characterize the cytoskeletons of growing sprouts as they emerge from ABC dendritic tips.

MATERIALS AND METHODS

Operations. Close axotomy of ABCs was performed as described (8) by hemisection of the hindbrain at a point less than 500 μm caudad of the rostralmost ABC. This left contralateral ABCs intact to serve as internal controls for effects of surgery (such as deafferentation, local trauma, and ischemia) and for any section-to-section variations in immunocytochemical processing.

Immunocytochemistry. Immunocytochemical analysis was performed on 6-μm transverse sections of paraffin-embedded lamprey brain and spinal cord. Brains were fixed by immersion of the entire head of anesthetized lampreys in Bouins' fixative for 6–12 hr at 4°C. These were then washed in 100 mM Tris buffer, dehydrated, impregnated with paraffin, and serially sectioned through the hindbrain. Sections were deparaffinized in xylene and then examined to determine if they contained sections through ABC somata and dendrites. They

Abbreviations: ABC, anterior bulbar cell; NF, neurofilament; MT, microtubule; Ac, acetylated; Tyr, tyrosinated; mAb, monoclonal antibody.

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were then rehydrated, and their endogenous peroxidase activity was quenched with 6% H_2O_2 in 80% methanol for 30 min before application of primary antibody (hybridoma supernatant without dilution unless otherwise noted) overnight at 4°C. Antibody staining was revealed with an ExtrAvidin–horseradish peroxidase kit (Sigma) by using secondary antibody concentrations, incubation times, and blocking and washing steps as recommended by the manufacturer. Diaminobenzidine (0.5 mg/ml) was used as the chromagen.

Antibodies. The anti-NF monoclonal antibodies (mAbs) employed in this study (RMO34, RMdO15, and RMO308) have been extensively characterized (17–20); and the specificity of RMO34 to highly phosphorylated NFs, of RMdO15 to nonphosphorylated NFs, and of RMO308 to phosphorylation-independent epitopes on NFs in the lamprey has been established (20). Three mAbs against α-tubulin were used to stain for total α-tubulin (mAb DM1A; ICN) and to differentiate between relatively stably polymerized MTs and labile MTs or free tubulin. A mAb against acetylated α-tubulin (mAb 6-11b-1; kindly provided by G. Piperno of Rockefeller University, New York) was used at a dilution of 1:5 (hybridoma supernatant) to label MTs that are stably polymerized (21, 22). A mAb against tyrosinated α-tubulin (mAb TUB-1A2; Sigma) was used (ascites, diluted 1:80) to identify tubulin that is not stably polymerized (23, 24). The ability of these mAbs to recognize lamprey tubulin specifically was confirmed on Western blots of lamprey brain cytosolic preparations run next to extracts of embryonic rat brain (data not shown).

Identification of ABC Somata and Dendrites. Normal ABC morphology has been described from both whole mounts and serial reconstructions of Lucifer yellow-injected cells (8, 9). ABC somata are located in the floor of the IVth ventricle at the level of the VIIIth nerve in the hindbrain. Most dendrites extend laterally and ventrally from the soma and thus are cut longitudinally when transverse sections of the brain are taken (see Fig. 1). ABC somata and dendrites could be identified in sections by their characteristic locations and cross-sectional appearance. Processes resembling dendrites that were not connected to somata in a given section (see Fig. 2 *A–C*) could usually be identified in adjacent sections containing their somata. Virtually all large processes extending radially through the hindbrain at this level are dendrites belonging either to ABCs or Mauthner neurons.

Identification of Dendritic Sprouts. Profiles of processes that met the following criteria were identified as dendritic sprouts: (*i*) they were located within a "sprout zone" (Fig. 1) lying within 30 μm of the pial surface of the brain on the cut side at the level of the ABC somata and 50 μm or more away from both the midline and the entry point of the VIIIth nerve and (*ii*) they were larger than the small, axially oriented processes that populate this region in intact brain. Identification of sprouts was done in deparaffinized, unstained sections. It is highly unlikely that sprouts emerging from the axon stumps of other axotomized giant neurons would be located in the sprout zone, as it is relatively distant from their axons (Fig. 1). Furthermore, Lucifer yellow fills of such neurons have not revealed axonal sprouts in this region (G.F.H., unpublished observation), whereas most ABC dendritic tips are located here and the sprouts that emerge from them tend to grow axially near the pial surface of the brain (8, 13). In some cases, dendrites in this region could be seen giving rise to sprouts (see Fig. 3*A*). The only processes other than ABC dendritic sprouts that might fit these criteria are dendritic sprouts from axotomized Mauthner neurons (whose response to close axotomy is similar to that of ABCs).

RESULTS

Immunostaining of Intact ABCs. Each of the mAbs used in this study had a characteristic staining pattern of ABC somata, dendrites, and axons in sections of lamprey brain and spinal cord. The results obtained by immunocytochemistry were in agreement with the known ultrastructure of the axons and dendrites of intact ABCs (14), in which MTs are most common in the soma and dendrites, and NFs predominate in the axon. The mAbs directed against acetylated (Ac), tyrosinated (Tyr), and total α-tubulin all stained ABC dendrites much more strongly than they did axons, whereas the reverse was true of the mAb directed against a phosphorylation-independent epitope on lamprey NF protein (RMO308). mAbs directed against phosphorylation sites clearly distinguished the axon from dendrites. The mAb directed against highly phosphorylated NFs (RMO34) stained only the axon, and the mAb against the dephosphorylated phosphorylation site (RMdO15) stained primarily the soma and dendrites. These patterns were identical to the ones reported for the same mAbs in lamprey by Pleasure *et al.* (20). mAbs to Ac- and Tyr-tubulin stained somewhat different patterns in the soma and dendrites, with Ac-tubulin staining (labeling stable MTs) strongest in the soma and proximal dendrites and Tyr-tubulin staining (labeling labile MTs) strongest in the distal dendrites. The mAb to total α-tubulin stained dendrites somewhat more intensely than the soma overall, with the strongest staining located at the periphery of the soma at the point where the major lateral and ventral dendrites arise. In the axon, all anti-tubulin mAbs stained with a fine punctate pattern (presumably of bundles of MTs in cross-section) that was distinctly weaker than the overall staining in ABC somata and dendrites. Normal patterns of ABC somadendritic staining by these mAbs are shown in control cells in Figs. 2 and 3.

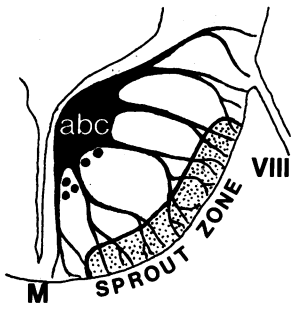

FIG. 1. Schematic diagram of ABC soma and dendrites (viewed as a transverse section at the level of the VIIIth nerve) illustrating the ABC dendritic field and the criteria used to identify dendritic sprouts after close axotomy of ABCs by a hemisection of the brain parallel to the plane of view. Higher order ABC dendritic branches have been omitted for clarity. Six-micrometer sections through the ABC dendritic tree (as shown in Figs. 2 and 3) generally did not include dendrites along their entire length, owing to oblique dendritic trajectories, although entire dendrites could usually be assembled in two to three adjacent sections. The stippled area (sprout zone) denotes the region in which swollen profiles were defined as dendritic sprouts. It was assumed that this sampling region [including most ABC dendritic tips, but distant from the trajectories of other giant axons (black circles near the ABC soma)] would prevent the misidentification of sprouts regenerating from the cut axons of other giant neurons as ABC dendritic sprouts. VIII, VIIIth nerve; M, midline. Dorsad is toward the top of the figure.

Effects of Close Axotomy on ABC Somata and Dendrites. Close axotomy resulted in rapid changes in the staining patterns described above. Fourteen out of 15 cells in seven animals examined between 2 and 6 days after close axotomy exhibited diffuse phosphorylated NF staining evenly distributed through their somata and dendrites (Fig. 2*A*). These same cells (in adjacent sections) showed a distinct increase in the level of Tyr-tubulin staining over that seen in the contralateral intact ABCs that served as controls (Fig. 2*C*). Furthermore, those axotomized cells that stained more

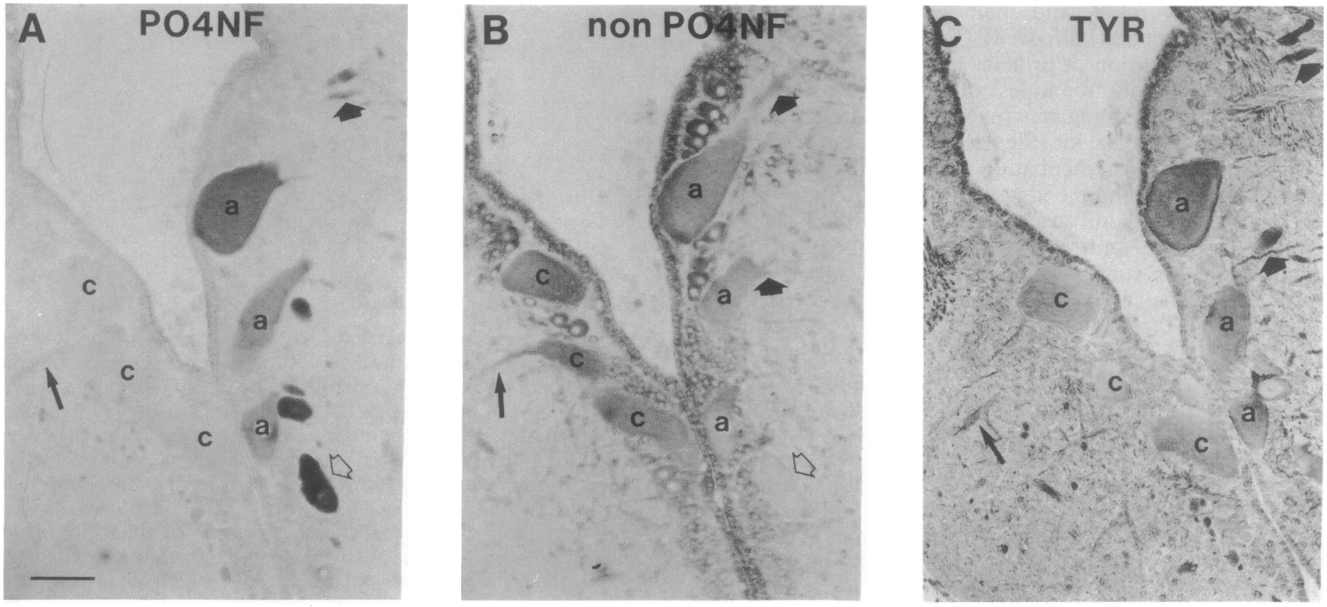

FIG. 2. Adjacent sections through the hindbrain of a lamprey fixed 6 days after hemisection of the right side, showing somata and dendrites of intact ABCs and a Mauthner neuron (all labeled c) and contralateral cells subjected to close axotomy (all labeled a). Sections were stained with mAbs recognizing highly phosphorylated NFs (PO4NF) (*A*), nonphosphorylated NFs (nonPO4NF) (*B*) and tyrosinated α-tubulin (TYR) (*C*). Note the correlation between axotomy and staining of the somata and dendrites for phosphorylated NFs, a slight reduction in staining for nonphosphorylated NFs, and increased staining for tyrosinated tubulin. Note also that those somata and dendrites staining most intensely with mAbs recognizing high phosphorylated NFs also have the strongest staining with mAbs recognizing tyrosinated tubulin. Narrow black arrows, dendrites of control cells; broad black arrows, dendrites of axotomized cells; hollow arrows, cut axonal stumps of other giant reticulospinal neurons. Note that the topmost broad black arrow in each panel labels a single dendrite passing through all three sections, which is attached to the topmost soma in *B*. (Bar = 50 μm.)

strongly for phosphorylated NF in a given section also showed stronger Tyr-tubulin staining (compare Fig. 2 *A* and *C*). A slight reduction in staining of nonphosphorylated NF was seen in most (10 of 14) of these cells (Fig. 2*B*). A reduction in Ac-tubulin staining was also seen in most ABCs (9 of 13) examined before 8 days postaxotomy. All of these changes were well established in 22 ABCs examined at times near the onset of sprouting (10–22 days postaxotomy; Fig. 3 *A–D*), by which time staining for total NF (RMO308) in the soma and dendrites had also increased over control levels (data not shown; stained in 7 of 9 cases). Total tubulin (DM1A) patterns in the somata and dendrites of six axotomized ABCs did not change systematically compared to controls during the first 16 days postaxotomy.

Twelve out of a total of 65 primary ABC dendrites stained with mAb directed against acetylated tubulin between 10 and 22 days after close axotomy showed a sharp localized loss of Ac-tubulin staining. Eight of these dendrites exhibited some type of abnormal gross morphology in the Ac-tubulin- stained section or in adjacent sections, such as a swelling (three cases; see Fig. 3 *D* and *E*) or gave rise to sprouts (five cases; see Fig. 3*D*). The other 4 dendrites showed no abnormalities in the Ac-tubulin-stained section but could not be traced to adjacent sections. None of the 53 primary dendrites that exhibited normal staining gave rise to swellings or sprouts in the same or adjacent sections. Normal staining was considered to be staining that was not clearly weaker than that of the rest of the cell. This correlation between abnormal dendritic morphology and localized loss of Ac-tubulin staining was highly significant ($P < 0.001$, χ^2 test).

Immunocytochemistry of Dendritic Sprouts. Swollen profiles began to appear among the dendritic tips of ABCs subjected to close axotomy between 10 and 16 days postaxotomy (Figs. 1 and 3). These correspond to the swollen tips of incipient dendritic sprouts identified in Lucifer yellow fills (8). Forty-one profiles were identified as dendritic sprouts of ABCs or Mauthner cells between 16 and 30 days after close axotomy by using the criteria described in *Materials and Methods* (Fig. 1). Each of these sprouts was identified in one or more sections stained with various mAbs. All 28 profiles identified as sprouts that were tested for highly phosphorylated NFs (RMO34) stained heavily. This was true of both large and small diameter profiles and thus differs from the situation found in intact axons, where only large diameter axonal profiles were found to stain with RMO34 (20). This staining was clearly stronger than that seen in the somata and dendrites of ABCs following close axotomy (Fig. 3*A*). In contrast, staining for unphosphorylated NFs (RMdO15) was very light in dendritic sprouts (all seven sprouts tested); this staining was even lighter than that seen in the dendrites of sprouting ABCs (Fig. 3*B*). Staining for total α-tubulin was generally light and often absent. In 21 of 22 profiles stained, Ac-tubulin staining was absent (Fig. 3*D*), while significant Tyr-tubulin staining was often present (9 of 17 profiles, see Fig. 3 *C* and *F*). However, Tyr-tubulin staining in sprouts was never as strong as that seen in ABC dendrites.

DISCUSSION

Early Somatic and Dendritic Changes Following Close Axotomy. An increase in somatic NFs is a common early response to axotomy in vertebrate neurons, especially in large cells, whose axons normally contain many NFs (1, 2). It is likely that this is due to a reduction in the rate of transport of NFs from the soma to the axon following axotomy (25, 26). An increase in the phosphorylation state of somatic NFs following axotomy has also been demonstrated in mammalian sensory and motor neurons by Goldstein *et al.* (3), who have suggested that this phosphorylation may cause the "backing up" of NFs into the soma, as it has been shown that highly phosphorylated NF proteins are transported slowly, if at all, relative to unphosphorylated forms (27) in mammalian optic nerve. In lamprey ABCs, we have found that an increase in the immunocytochemical staining for total NFs is accompa-

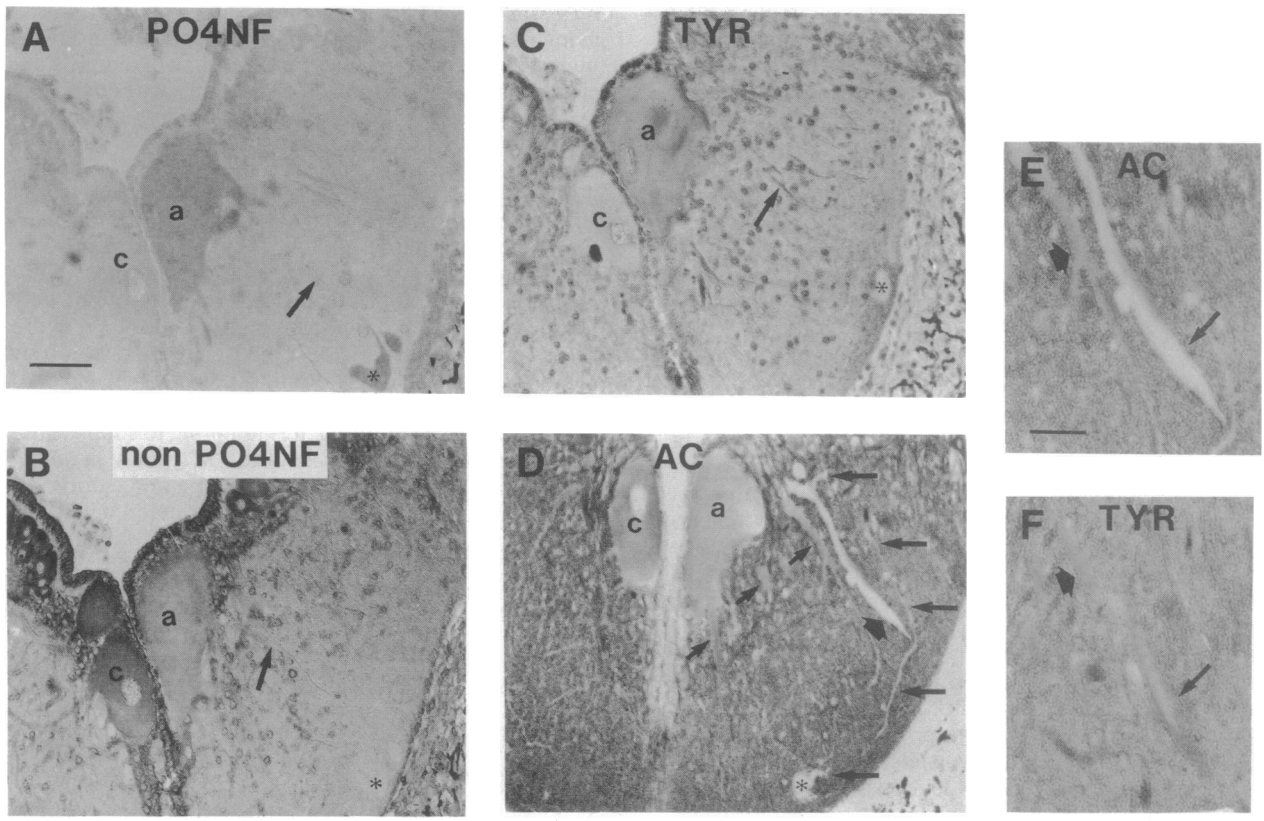

FIG. 3. (*A–D*) Sections showing ABC somata and dendrites after close axotomy of cells on the right (labeled a) 16 days previously. The cells on the left (labeled c) were left intact to serve as controls. Axotomized ABCs give rise to dendritic sprouts (asterisks). Sections were stained for highly phosphorylated NFs (PO4NF) (*A*), nonphosphorylated NFs (nonPO4NF) (*B*), tyrosinated (labile) MTs (TYR) (*C*), and acetylated (stably polymerized) tubulin (AC) (*D*). Sections in *A–C* are adjacent sections taken of a single pair of ABCs; the section in *D* is from a different lamprey. (*E*) Swollen dendrite in *D* (stained for acetylated tubulin) at higher magnification. (*F*) Adjacent section stained for tyrosinated tubulin at the same magnification as in *E*. (*A–D*) All of the sprouts indicated by asterisks arise from the dendrites of the cells shown (arrows); in *D* the course of the dendrite is traced (long, narrow arrows) with the missing portions of its trajectory present in adjacent sections. Note that the somata and dendrites of axotomized ABCs show the same changes in staining patterns relative to controls as ABCs at earlier times postaxotomy (Fig. 1). The sprout stains more intensely for phosphorylated NFs and less intensely for nonphosphorylated NFs and tyrosinated tubulin than the soma and dendrites of the parent ABC. (*D*) Overall staining for acetylated tubulin in the axotomized cell is slightly reduced relative to control levels, and a swollen dendrite (broad arrow) is nearly devoid of staining for acetylated tubulin along its entire length. Other primary dendrites (arrows) of the axotomized cell resemble the control cell. A dendritic sprout tip (asterisk) arising from an adjacent dendrite (long, narrow arrows) is also devoid of staining for acetylated tubulin. (*E*) Note that the dendrite lacking staining for acetylated tubulin (arrow) does not taper normally as does a nearby dendrite (broad arrow) but shows a pronounced swelling, a preliminary stage in dendritic sprouting (8). This dendrite stains unevenly but strongly with mAbs against tyrosinated tubulin in an adjacent section (*F*), suggesting that the original stable MTs in this dendrite have been displaced and destabilized. (*A–D*, bar = 50 μm; *E* and *F*, bar = 25 μm.)

nied by a shift from dephosphorylated to highly phosphorylated NFs in the soma and dendrites at early times postaxotomy. Even at the earliest times postaxotomy, we never saw a staining gradient for highly phosphorylated NFs between the soma and dendrites; the intensity of the staining increased uniformly with time postaxotomy. This suggests that NFs were probably first transported into the dendrites and then phosphorylated in place, since assembled, phosphorylated NFs would travel extremely slowly and produce a somatofugal staining gradient at early times postaxotomy. It is thus possible that the primary disturbance caused by axotomy is the abnormal distribution of NF kinases, which, by phosphorylating and immobilizing NF protein in the soma and dendrites, could account for the increases in total and phosphorylated NFs in these locations, as well as for the coincident loss of dephosphorylated NFs.

An important feature of the changes in NF phosphorylation and MT stability observed in this study is the correlation between the increase in NF phosphorylation and the changes in Ac- and Tyr-tubulin staining in ABC somata and dendrites. The increase in Tyr-tubulin staining is likely due to the destabilization of existing MTs, as it is temporally correlated with a loss of Ac-tubulin staining. Furthermore, preliminary results of an ultrastructural study of ABC dendrites at early times following close axotomy (G.F.H. and M. J. Cohen, unpublished results) indicate a general reduction in the number of MTs. This suggests that some existing somadendritic MTs are actually lost and are not just becoming more dynamic (although this may also be occurring). These observations, taken together, therefore suggest that the appearance of highly phosphorylated NFs may be related to the destabilization and eventual loss (14) of most dendritic MTs following close axotomy. It is possible that the increase in ectopic NFs might destabilize MTs via the increased competition from the NFs for microtubule-associated protein binding, as the ability of NFs to destabilize MTs in this manner has been demonstrated *in vitro* (28).

A selective loss of Ac-tubulin staining was observed in sprouting dendrites relative to other dendrites in the axotomized cell (Fig. 3 *D* and *E*). Since it is known that only a small subset of ABC dendrites gives rise to sprouts at this time following close axotomy (8, 9), it is likely that a loss of acetylated tubulin generally occurs in sprouting dendrites, either as a consequence of, or as a preliminary to, sprouting. The loss of Ac-tubulin staining from four dendrites that did not exhibit abnormal morphology may be due to one of

several reasons: (*i*) local loss of Ac-tubulin staining may normally occur before changes in dendritic morphology after close axotomy, (*ii*) the affected dendrites may exhibit abnormal morphology elsewhere in their trajectories that was not detected in adjacent sections, and (*iii*) dendritic swellings and/or sprouting may not always be associated with the selective loss of AC staining. However, the highly significant statistical correlation ($P < 0.001$) between these two events does indicate that they are related in some important way.

Immunocytochemistry of Dendritic Sprouts. Dendritic sprouts from ABCs following close axotomy exhibit several characteristics that are atypical of the "conventional" growth cones seen in cultured neurons and developing neurites *in vivo*. These are (*i*) the presence of phosphorylated neurofilaments as the dominant cytoskeletal element, (*ii*) the virtual absence of tubulin from at least some sprout tips, and (*iii*) the lack of filopodia and balloon-like cross-sections of most sprouts. Conventional growth cones, on the other hand, lack neurofilaments entirely (15, 16), contain significant amounts of tubulin, and generally have a flattened appearance with large numbers of filopodia when engaged in active growth. This unusual appearance and composition of ABC dendritic sprout tips raises the question of whether these sprouts elongate by the same mechanisms that underlie growth cone-mediated growth.

In this study, we have found that early dendritic sprouts have neurofilamentous cytoskeletons at a time when they are elongating steadily (8, 9). This is surprising as NFs (especially phosphorylated NFs) are often considered to be inert structures that are expressed late in development and serve an exclusively structural role in maintaining neuronal form (29, 30). Furthermore, the virtual absence of tubulin in some sprouts calls into question the ability of the cell to transport materials to the growing tips, since vesicular transport has generally been found to be dependent on MT-mediated transport. However, it is possible that a few MTs are present in all sprouts but are not detected by light microscopic immunocytochemical analysis.

Previous light microscopy and ultrastructural studies of ABC dendritic sprout tips (8, 9, 14) did not indicate the presence of filopodia or lamellopodia-like structures, except in the special cases when they were flattened onto the ventral margin of the brain (13). This raises the question of whether the ability of these sprouts to elongate is dependent on growth cone motility. A number of *in vitro* studies have shown that growth cones advance by generating tension on adhesive surfaces (31, 32) and that neuritic outgrowth *in vitro* requires adhesive substrates (33, 34). On the other hand, both *in vivo* and *in vitro* experiments have shown (35, 36) that abolition of filopodia by the administration of cytochalasin does not always block axonal elongation, although guidance is impaired. Letourneau *et al.* (37) have shown that extensive neurite outgrowth can occur *in vitro* in the absence of both filopodia and contact with an adhesive surface. The possibility that the elongation of ABC dendritic sprouts may be due to protrusive forces exerted by the polymerization or extrusion of NFs rather than by growth cone-generated tension should therefore be considered.

Summary and Conclusions. Close axotomy of giant reticulospinal neurons in the central nervous system of the larval lamprey results in an increase in immunocytochemical staining for NFs in the soma and dendrites, together with a major increase in their degree of phosphorylation. This is spatially and temporally coupled with a large increase in Tyr-tubulin staining and a decrease in Ac-tubulin staining. We interpret this as the destabilization of existing dendritic MTs. The destabilization and loss of acetylated (presumably stable) MTs was most marked in those dendrites that were seen giving rise to sprouts. The dendritic sprouts themselves have the highest level of staining for phosphorylated NFs and the least staining for any form of tubulin. Although it is not possible to assign causal relationships between any of the consequences of close axotomy listed above based on the data presented, it should be noted that the increase in phosphorylated NFs, the decrease in unphosphorylated NFs, and the destabilization of MTs in the dendrites all occur at early times after close axotomy in advance of the onset of dendritic sprouting. This is consistent with the possibility that these events determine the distribution of sprouting between the dendritic tips and the axon stump and thus may be key factors in the failure of these neurons to maintain normal polarity following close axotomy. In light of the close spatial association between the loss of stable MTs and the occurrence of dendritic sprouting, it is tempting to suppose that the destabilization and loss of dendritic MTs may in some way permit NF invasion of the dendritic tree and ectopic axonal regeneration from the most severely affected dendrites.

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