by varying the test stimulus, evaluating other motor responses, or by studying response decrement to repetitive stimulation. We suggest that these studies be directed to the broad topic of the developing central nervous system and not be restricted conceptually to the issue of hearing alone.

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Extensive Dendritic Sprouting Induced by Close Axotomy of Central Neurons in the Lamprey

Abstract. *Massive dendritic sprouting was induced in identified giant reticulospinal neurons of the lamprey by axotomy close to the soma. An axonal lesion slightly farther from the cell body induced new growth from both dendrites and axon. The amount of new growth per cell was the same whether it originated from the dendrites alone or from axonal and dendritic compartments. The location of the axonal lesion therefore determines where, in the neuron, membrane is inserted to produce the new neurites. The dendritic tree of a differentiated vertebrate central neuron was shown to have sufficient plasticity to extend new growth for several millimeters beyond the normal dendritic domain.*

The characteristic form of a mature neuron results from selective growth in different regions of the cell leading to the generation of an axon and dendrites. The mechanisms underlying this selective growth can be studied by examining the location and extent of sprouting after axotomy of mature neurons. Generally, the most prominent response to axotomy is the partial or complete regeneration of the severed axon, which may be accompanied by relatively minor changes in the dendritic tree (*1–3*). However, in recent invertebrate studies, axotomy close to the soma induced profuse intraganglionic sprouting with relatively little axonal regeneration into the connectives (*4*). In the cricket, the sprouting definitely originated from the dendritic tree. We now report that "close" axotomy (< 500 μm) from the soma of identified central neurons in a primitive vertebrate, the sea lamprey, induced extensive dendritic sprouting while greatly decreasing axonal regeneration. Axotomy farther from the soma (1000 to 1400 μm) results in less dendritic and more axonal sprouting than close axotomy, with the total amount of growth induced by the two types of lesion being roughly equal. These results suggest that close axotomy causes a shunting of new growth into the dendrites and away from the axon stump without altering the total extent of regenerative sprouting.

Larval sea lampreys (*Petromyzon marinus*) 9 to 13 cm long were anesthetized by immersion in a saturated aqueous benzocaine solution. An incision was made dorsally across the skull at the level of the rostral midbrain. The top of the cartilaginous skull and the choroid plexus were then partially freed and reflected caudally to expose the hindbrain. A partial hemisection of the brain was made at one of two locations in the hindbrain with a No. 11 scalpel blade (Fig. 1A), and the choroid and skull were then replaced. No suturing was necessary. For distant axonal transection in the spinal cord, a scalpel blade was used to sever the cord with a dorsal incision. All lesions extended through the entire dorsoventral dimension of the brain or spinal cord. The animals were allowed to recover in lamprey saline (*5*) for 3 days, and were gradually returned to well water by 6 days where they were kept at 15°C until examined histologically.

Intracellular staining with Lucifer yellow (*6*) was used to study the morphology of normal and axotomized cells. Somata of the three most anterior pairs of large bulbar Müller cells (*7*) (anterior bulbar cells or ABC's; see Fig. 1) were

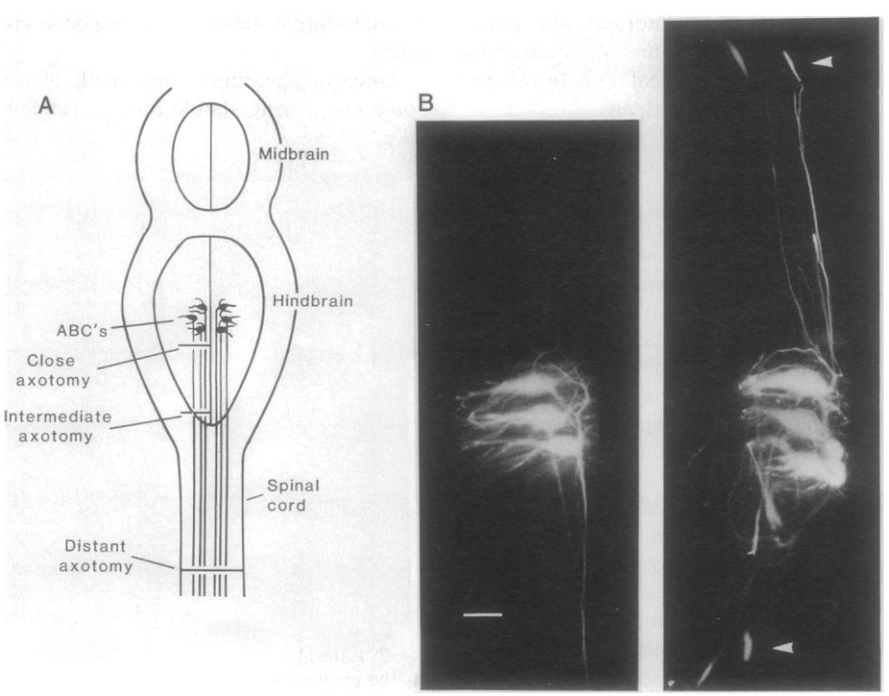

Fig. 1. (A) Diagram showing the lamprey brain in dorsal view with the cell bodies of the three most anterior pairs of bulbar Müller cells (ABC's) in the hindbrain and their axons extending caudally down the spinal cord. In various experiments, these neurons were axotomized at the three locations indicated. (B) Whole mounts of lamprey brain showing three ABC's from a normal animal (left) and from another animal 49 days after close axotomy (right). Arrows indicate sprouts with swollen tips extending from the dendrites of the axotomized cells. No axonal regeneration is apparent. Cells were stained by intracellular injection of Lucifer yellow. Scale bar, 100 μm.

iontophoretically injected with 5 percent Lucifer yellow in 5 m*M* LiCl. Glass micropipettes with a resistance of 20 to 50 megohms were used for the injections. Identification and impalement of ABC's were accomplished by visual guidance under a dissecting microscope and the location of the lesion with respect to ABC somata was recorded for each animal. Brains injected with Lucifer yellow were prepared for conventional fluorescence microscopy in whole mount by fixation in 4 percent formaldehyde followed by ethanol dehydration and clearing in methyl salicylate.

Normal ABC's have a well-defined dendritic structure with one or two large, profusely branched dendrites extending dorsolaterally along the floor of the fourth ventricle for approximately 150 μm before turning ventromedially. Other primary dendrites project ventrally and anteriorly from the medial aspect of the soma and caudolaterally from the periaxonal region. Almost all dendrites terminate within 300 μm of the soma (Figs. 1B, 2, and 3). Eighty-seven intact ABC's were examined in 47 animals, and the dendrites of all cells conformed to this basic pattern.

ABC's axotomized within 500 μm of the soma began showing dendritic changes soon after axotomy. Fifteen of 33 cells examined 5 to 12 days after axotomy showed swollen dendritic tips resembling growth cones, which generally occurred within the confines of the normal dendritic tree. Twelve of 17 cells examined 23 to 27 days after axotomy showed sprouts extending beyond the normal dendritic tree (Fig. 2b). The extent of dendritic sprouting was greatly increased in cells examined 50 and 80 days after axotomy, with some sprouts attaining lengths of 3 mm or more (Fig. 1B and Fig. 2, c and d). Dendritic sprouts tended to grow along the rostro-caudal axis and next to the ventricular and pial surfaces of the brain and rarely crossed the midline. The mean total sprout length per cell increased linearly with time up to at least 80 days after axotomy (Fig. 3).

The response of proximal axon stumps to close axotomy was markedly different from that of dendrites. Fewer than 10 percent of the ABC's subjected to close axotomy appeared to have any axonal sprouts. Axon stumps were completely missing in more than half of the cells studied beyond 25 days after axotomy. When stumps were present, they appeared as large, straight, unbranched processes extending caudally along the midline from the soma, and they were usually clearly distinguishable from normal dendrites or dendritic sprouts. When an axon stump was not visible, it was sometimes difficult to determine whether sprouts near the site of the old axon were of dendritic or axonal origin. However, even when all processes that could have originated from the remains of an axon stump were considered to be axonal sprouts, there was less growth from the axon than from the dendrites (Table 1).

"Intermediate" lesions slightly farther from the soma (1000 to 1400 μm) resulted in sprouting from both axon and dendrites in all of 27 ABC's in nine animals examined 47 to 54 days after cutting the axons. The distribution of sprouting was different from that caused by close axotomy, with significantly more growth

Table 1. Comparison of the extent of axonal and dendritic sprouting in cells subjected to close (200 to 500 μm) versus intermediate (1000 to 1400 μm) axotomy. The significance of differences between these groups was calculated on a per-animal basis by use of a Mann-Whitney *U* test. The total sprout length per cell was not significantly different in the two groups even though the apportionment of new growth in dendritic and axonal compartments differed greatly. Numbers in parentheses are percentages; N.S., not significant.

Distance of axotomy from soma (μm)	Mean summed sprout length per cell (μm)			Number of cells	Number of animals
	Axonal	Dendritic	Total		
200 to 500	463* (12)	3401 (88)	3864 (100)	21	13
1000 to 1400	2798 (72)	1108 (28)	3906 (100)	27	9
	$P < 0.01$	$P < 0.01$	N.S.		

*All sprouts arising from the periaxonal region were counted as axonal sprouts.

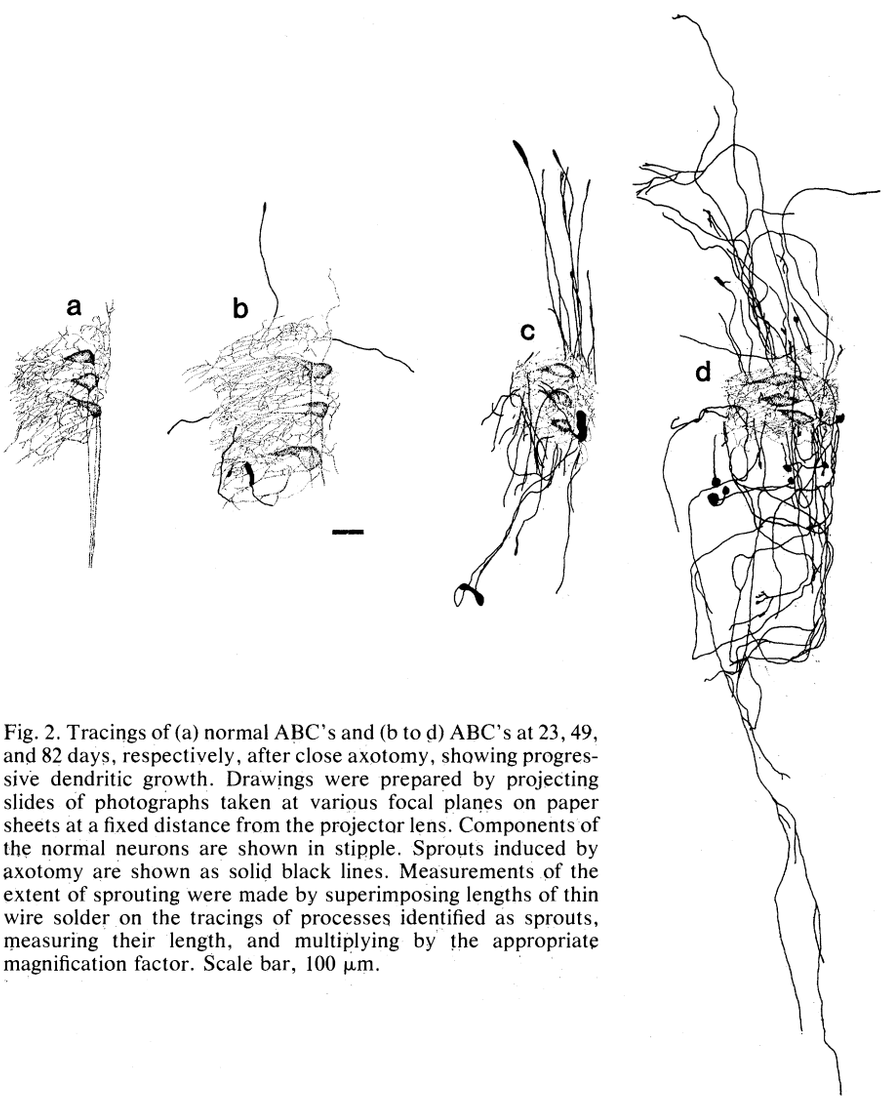

Fig. 2. Tracings of (a) normal ABC's and (b to d) ABC's at 23, 49, and 82 days, respectively, after close axotomy, showing progressive dendritic growth. Drawings were prepared by projecting slides of photographs taken at various focal planes on paper sheets at a fixed distance from the projector lens. Components of the normal neurons are shown in stipple. Sprouts induced by axotomy are shown as solid black lines. Measurements of the extent of sprouting were made by superimposing lengths of thin wire solder on the tracings of processes identified as sprouts, measuring their length, and multiplying by the appropriate magnification factor. Scale bar, 100 μm.

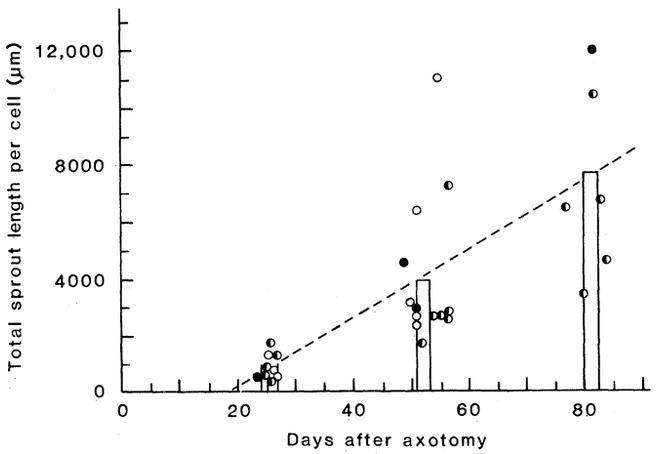

Fig. 3. The mean value for the summed sprout length of all injected cells in each animal was calculated and the results are shown in a scatter plot. Each circle represents an animal with one (○), two (◐), or three (●) filled cells. In all, 16 cells in nine animals were examined at approximately 25 days after axotomy, 23 cells in 13 animals at 50 days, and 14 cells in six animals at around 80 days after axotomy. Mean total sprout length per cell was calculated for each of the above times (bars). The overall mean rate of sprouting was approximately 100 μm/day per cell over the time period examined.

originating from the axon stump (Table 1). With the intermediate lesion, the mean sprout length per cell from dendrites and axon was approximately equal to that of neurons subjected to close axotomy, in which the sprouting is predominantly from the dendrites alone (Table 1).

The response to "distant" axotomy in the spinal cord at a point 1.5 to 2 cm from the soma was examined in 20 ABC's 50 to 56 days postlesion. Little or no dendritic sprouting was present in these cells. Other studies (*8–11*) have shown that Müller cell axons severed in the spinal cord frequently regenerate for several millimeters caudal to the lesion. This, together with the finding that the proximal axon stump first dies back about 2 mm before regeneration begins (*12*), indicate that the extent of axonal sprouting after distant axotomy is roughly comparable to the amount of dendritic growth induced by close axotomy.

Generally, no marked differences in the morphology of dendritic and axonal sprouts were seen in Lucifer yellow–stained whole mounts. Both types of sprouts bore a closer resemblance to axons than to dendrites in that they tended to grow linearly for long distances and were relatively sparsely branched.

Homologous ABC's contralateral to cells axotomized in the hindbrain were undamaged and served as controls. No sprouting was ever observed from dendrites or axons in these cells (Figs. 1B and 2). This probably rules out the involvement of systemic humoral agents in the dendritic sprouting brought about by close axotomy. Furthermore, partial amputation of ABC lateral dendrites without axotomy appears to be ineffective in provoking sprouting from the remnant of the dendritic tree (*13*). This suggests that deafferentation does not play a major role in this dendritic growth response. Therefore, we conclude that the axotomy itself is primarily responsible for the growth of the dendrites evoked by axotomy close to the soma.

Our findings indicate that the apportionment of sprouting between axon and dendrites after axotomy of ABC's is determined by the distance of the axonal lesion from the soma. The total amount of growth induced by axotomy at various locations appears to remain constant. This suggests that one effect of close axotomy is to divert to the dendrites new growth otherwise destined for the axon stump. The gross morphological similarity between dendritic and axonal sprouts is consistent with this hypothesis. Whether dendritic sprouts have typical axonal features such as action potential propagation or presynaptic terminals remains to be determined.

Several different mechanisms might be involved in mediating the dendritic growth associated with close axotomy. The length of viable proximal axon stump has been proposed as a crucial factor in determining the distribution of sprouting induced by axotomy (*4*). This in turn has been related to the massive injury current that enters the cut face of the transected lamprey spinal cord (*14*). Sodium and calcium ions are the major components of this injury current. This influx of cations, by disrupting elements of the cytoskeleton, may be responsible for the dieback of the proximal axon stump in the transected giant axons of the lamprey cord. The axonal dieback can frequently extend as much as 2 mm proximal to the lesion (*12*). Therefore, such a mechanism would be sufficient to eliminate completely the proximal axonal stump, as frequently happens, when the lesion is close to the soma (< 500 μm). The degenerated proximal axonal stump may then no longer provide a suitable target for newly synthesized membrane, which would then be shunted into the dendritic tree to produce the observed sprouting. There may also be a change in the intracellular transport system responsible for distributing membrane within the cell. The effect of cations, in particular calcium, on the cytoskeletal elements involved with intracellular transport is well known (*15–17*). A change in microtubule organization has been reported in other central neurons after close axotomy and subsequent dendritic sprouting (*18*). It may be that the influx of cations from the injury current of the closely transected central nervous system of the lamprey is also involved with altering the cytoskeletal elements in the Müller cell somata after close axotomy. This in turn could result in a rerouting of the newly synthesized membrane from the degenerated axonal target into the dendritic tree.

Previously reported changes in the dendritic morphology of axotomized vertebrate neurons appear to differ fundamentally from our results. The dendritic retraction and reexpansion after axotomy in rat hypoglossal motoneurons is thought to be correlated with the loss and regaining of specific postsynaptic contacts (*3*). In ABC's subjected to close axotomy, dendritic growth is probably not triggered by synaptic reconnection of the regenerating axon to its original target, since the axon frequently undergoes complete retrograde degeneration and fails to regenerate. However, it is the quantity of dendritic sprouting that sets our results most clearly apart from the results obtained in other vertebrate systems. The induced dendritic growth observed in other vertebrate preparations has occurred mainly within the limits of the normal dendritic field (*2, 3, 19*). In contrast, dendritic sprouts from ABC's subjected to close axotomy extend for millimeters beyond the usual dendritic domain of these neurons into alien regions of the brain. Our results with close axotomy indicate that a differentiated vertebrate central neuron retains a great potential for new dendritic growth, which may lead to extensive reorganization of neuropil within large areas of the brain.

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Paedomorphosis and Neoteny in the Pygmy Chimpanzee

Abstract. *The strongly paedomorphic skull form in the pygmy chimpanzee results from the heterochronic process of neoteny. This cranial paedomorphosis and neoteny in* Pan paniscus *may be related to reduced sexual dimorphism in morphology and behavior. The interspecific differences in form result from shifts in the rate and timing of similar patterns of development.*

The nature of the morphological differences between the "pygmy" and "common" chimpanzees and the meaning of these differences for our understanding of ape and human evolution have long been of interest to primatologists. Early investigators (*1, 2*) suggested that the pygmy chimpanzee (*Pan paniscus*) represented a "dwarfed, paedomorphic" form of chimpanzee (*1, 2*); later others (*3, 4*) also invoked paedomorphosis and neoteny to account for the differences between the chimpanzee species, but detailed studies to support these hypotheses have not been made.

I have integrated studies of allometry [size and shape relations (*5*)] and heterochrony [shifts in the timing of developmental patterns (*6–9*)] in an analysis of chimpanzee (and gorilla) morphology (*9–16*). My results indicate that most of the shape differences between adult pygmy and common chimpanzees are allometric because of "ontogenetic scaling." That is, the primary differences in shape result from a simple extension of common growth allometries to different terminal sizes (Fig. 1a) (*17*). This has been found to generally hold within the skull (*13–15*), within the trunk (*14*), and within each limb (*10*), but not in a comparison of hind limb length relative to trunk or forelimb length (*10, 14*). I now discuss new findings concerning these issues, resulting from more detailed comparisons among various body regions in the two species of chimpanzees.

Although ontogenetic scaling of proportions holds for almost all comparisons within the major body regions of the head, trunk and forelimbs, and hind limbs, the pygmy and common chimpanzees clearly do not fall along a common ontogenetic trajectory when comparisons are made between these major body regions. In fact, the data show a "gradient" of differential size (and correlated shape) change among the major body regions in a comparison of *P. paniscus* with *P. troglodytes* chimpanzees (*18*) (Table 1). Adult pygmy chimpanzees do not have overall proportions that match any single ontogenetic stage in *P. troglodytes*. Rather, given a large-to-small vector of size change, the skull is most strongly reduced in size, the forelimbs and body trunk are somewhat reduced, and the hind limbs are not reduced at all (*19*). The opposite changes characterize a small-to-large vector of size increase (that is, *P. paniscus* to *P. troglodytes*) (Fig. 1B) (*20*). A comparison of the ontogenetic allometries of maximum skull length against head-and-trunk length in the chimpanzee reveals a strong divergence of the growth trajectories; at a given body size, *P. paniscus* has a smaller overall skull size than *P. troglodytes* (Fig. 1C) (*21, 22*). Because of the strong ontogenetic scaling of cranial growth patterns, the smaller skull size in the

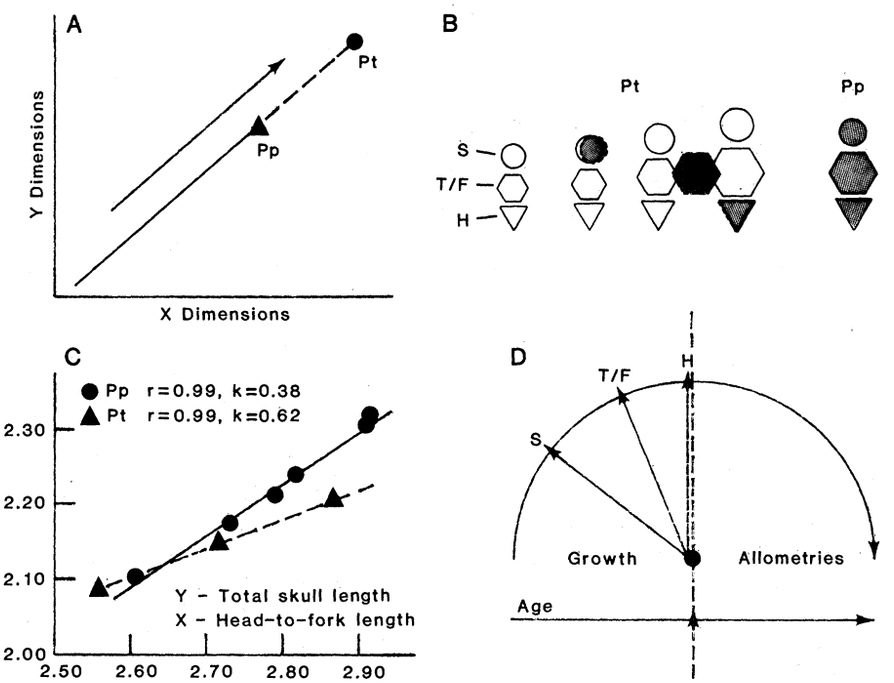

Fig. 1. (A) Paedomorphosis in *Pan paniscus* adults and the extension of common growth allometries to larger sizes in *Pan troglodytes* adults. (B) Relative size of the skull (*S*), trunk and forelimbs (*T/F*), and hind limbs (*H*) in adult *Pan paniscus* (shaded symbols) when placed on an ontogenetic sequence of infant, juvenile, subadult, and adult *Pan troglodytes* (open symbols). (C) Skull length (in millimeters) is plotted against a measure of trunk length (in millimeters) in ontogenetic sequences of the chimpanzees. The lower slope in the pygmy chimpanzees ($k = 0.38$; standard deviation, 0.01) compared to the the common chimpanzee ($k = 0.62$; standard deviation, 0.03) reflects slowed growth of the skull relative to overall body size, yielding paedomorphosis via neoteny. (D) Gould's (*7*) "clock model" of heterochrony, where the curved trajectory represents differential travel along common growth allometries. If the dashed vertical line is taken as the condition in adult *Pan troglodytes*, the position of the clock's "hands" for the skull, trunk and forelimbs, and hind limbs indicates the relative position of adult *Pan paniscus* (*17–22*); compare to (B).