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Trophic Regulation of Nerve Sprouting

Neuron-target interactions and spatial relations control sensory nerve fields in salamander skin.

J. Diamond, E. Cooper, C. Turner,* Lynn Macintyre

Studies on developing nervous systems have revealed that nerve cells can change their shape by sprouting axonal branches and reabsorbing old ones. The physiological connections which these branches make with their target cells are the basis for the establishment of circuitry in the nervous system. In studying nerve sprouting, we need to consider both the stimulus for its initiation and the way it is controlled. This control applies to the area over which endings are distributed and to the density of the endings within it. These two parameters, density and area, define the terminal field of a nerve. The control of sprouting then is a principal means of determining how nerve fields develop. This development could be regulated entirely by a rigid genetic program intrinsic to either the target tissue or the nerve, but a potentially more interesting mechanism would have a competence to respond to internal and external environmental demands. If so, then the dynamic regulation of terminal fields, including sprouting and possibly regression of endings, may be a normal feature of both central and peripheral neurons, even in the mature organism. This article deals with investigations of the regulation of terminal fields in a readily accessible peripheral system. It appears that an interaction between the nerve and the target tissue controls the density of the endings, while the area of a terminal field is more determined by spatial relations.

Sprouting During Development

In his observations on the genesis of epithelial innervation, Ramón y Cajal detected an important influence from the target tissue (*1*). He observed that the incoming fibers often grow relatively long distances to reach the epithelial tissues, but only after arriving at them do the nerves start sprouting collateral branches, each growing to a territory devoid of nerves. This sprouting eventually stops, and Ramón y Cajal noted the absence both of any vast aneuritic spaces and of any excessive collection of nerve fibers. He suggested that there are growth-pro-

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moting influences emanating from the target tissue, and that these influences ultimately become neutralized in some way by factors released from the nerves themselves. Speidel's visual observations on the living nerves growing into the transparent tail of the tadpole directly supported Ramón y Cajal's suggestion (*2*). In addition, he noticed that some of the collateral branches produced by the cutaneous nerves sprouted inappropriately into deep tissues. These were subjected to continual remodeling; some would suddenly change their direction, sometimes first retracting for variable distances, to establish connections with the skin successfully, while others eventually stopped elongating and degenerated completely.

The studies of Speidel and Ramón y Cajal revealed that collateral sprouting during development occurs almost entirely in the vicinity of the end organ. A later study by Fitzgerald on the primary innervation of the epidermis of the pig's snout indicated more convincingly the source of the sprouting stimulus (*3*). In this organ the number of dermal axons present at birth remains constant, but they continually sprout branches which penetrate to the epidermal ridges. Fitzgerald observed that the number of these endings increased in direct proportion to the increase in number of epidermal ridges after birth, suggesting that it is indeed the epidermis which provides the stimulus for the dermal axons to sprout.

Sprouting After Partial Denervation

Nerve sprouting occurs not only during primary development but also during maturity. There is evidence which suggests the possibility that both peripheral and central nerve endings are not static, but that new endings may be forming as others degenerate (*4*). However, the most striking demonstration of the ability of mature nerves to sprout is that which occurs when adjacent ones are cut; this "denervation sprouting" occurs in virtually all nerves, certainly in those of vertebrates. Speidel demonstrated this phenomenon by directly observing intact axons 3 days after sectioning one of the nearby cutaneous nerves of the tadpole tail. Weddell, Guttmann, and Gutmann (*5*), using behavioral and histological techniques, reported similar evidence for collateral sprouting of sensory nerves in the rabbit. Collateral reinnervation of partially denervated skeletal muscle has been quantitatively demonstrated, both histologically as well as functionally, by numerous workers, including Edds, Weiss, Hoffmann, and Van Harreveld (see *6*). In studying the autonomic nervous system of cats, Murray and Thompson (*7*) detected sprouting of preganglionic fibers after section of adjacent ones supplying the superior cervical ganglion. This work has been confirmed and extended (*8*). In man, the recovery of function after section of certain peripheral nerves and during some degenerative nerve conditions is best explained by collateral sprouting of the remaining fibers (*9*).

Although often more difficult to demonstrate, axonal sprouting occurs also in the mature central nervous system (CNS) of both mammals and lower vertebrates (*10*). The first clear demonstration of its occurrence in mature mammals was in the well-known experiments of Liu and Chambers (*11*). They sectioned all but one of the dorsal roots on the left side in the cat and, by histological techniques, showed that after 6 months the spinal ramifications of the remaining root extended further up and down the cord than did those of the corresponding root on the opposite side. In a comparable study on the rat, Goodman and Horel (*12*) demonstrated the sprouting of retinal projections in some visual nuclei after they were partially denervated by section of the visual cortical efferent fibers. Both of these pioneering studies were based on light microscopy and did not indicate whether new synapses formed. That collateral sprouts can form synapses was convincingly shown by Raisman (*13*), who studied the septal nuclei with the electron microscope; he showed that after lesions of the medial forebrain bundle, fimbrial fibers (which normally end on dendrites of septal cells) sprouted to form new synapses on some of the vacated sites on the somata of the cells. Although morphologically they looked normal, it was not possible to show whether these new synapses were functional. The occurrence of collateral sprouting in the mammalian CNS is now well established (*14*). Recent studies suggest that new collateral sprouts in the hippocampus and in the red nucleus do indeed make functional contacts (*15*).

Sprouting Without Denervation

The exact cause of "denervation sprouting" has always been a mystery. When nerves are cut, either in the central or peripheral nervous system, the part distal to the cell body degenerates and is removed by phagocytic activity involving a variety of other cell types. On the assumption that these nonneural cellular responses are triggered by products of nerve degeneration, it has long been thought that the stimulus for the nearby intact axons to sprout was of similar origin, even though attempts to obtain direct evidence for this have been unsuccessful (*16*).

A most striking example of adult nerve sprouting without nerve degeneration is that of Olsen and Malmfors (*17*), who showed that a piece of iris which had been deprived of its nerves 3 months earlier, when transplanted into the anterior chamber of the eye evokes collateral branching from the intact sympathetic nerves of the host. Some influence from the target tissue [perhaps nerve growth factor (*18*)] is clearly implicated, as Olsen and Malmfors suggested. The observations by Duchen and Strich (*19*) of sprouting of motor axons treated with doses of botulinum toxin, which prevented the release of acetylcholine but did not visibly cause nerve degeneration, do not support the "products of degeneration" hypothesis. More recently Aguilar, Bisby, Cooper, and Diamond (*20*) provided evidence suggesting that factors in living axons regulate nerve sprouting. In these experiments one of the three nerves to the hind limb of the salamander was briefly (30 minutes) exposed to a concentration of colchicine that interrupted fast axoplasmic transport, without noticeably interfering with the ability of the nerves to signal sensory information or drive muscles. After the colchicine treatment the peripheral fields of the two adjacent untreated nerves to the limb enlarged in area, as they did in experiments in which the nerve was sectioned rather than treated with colchicine (*21*).

In order to exclude the possibility that a scattered degeneration of some of the terminals of the colchicine-treated nerve may have occurred, we have now investigated the density of the mechanosensory endings in addition to the area over which they spread. To measure density, we recorded the mechanical stimulus required to evoke an afferent impulse at each of a large number of points on the skin, and made an appropriate analysis of the distribution of the thresholds. This analysis (*22*) gives the density of the touch receptors, and the method provides a very sensitive measure of receptor function. The validity of the analysis has now been shown by a direct correlation of physiologically identified "touch spots" with morphologically demonstrated sensory terminal processes; each mechanoreceptor is associated with a single Merkel cell (*23*). When an appropriate dose of colchicine was used, the number of mechanosensory endings of

the treated nerve was unchanged at a time when the adjacent nerves sprouted (Fig. 1B). From 5 to 6 days after nerve section the mechanical threshold needed to evoke an action potential rose markedly, and total insensitivity developed a day later, even though the axons usually were able to respond normally to electrical excitation. The results demonstrate that the effects of the colchicine were not due to nerve degeneration (*24*). Nor did the drug cause sprouting by a direct action on the skin; after tritium-labeled colchicine was used to treat the nerve, the small amount of systemically distributed label was always equal in the skin of both hind limbs, although the sprouting was on one side only (*24*). We conclude therefore that colchicine was effective in initiating sprouting in untreated nerves because of its interference with axonal transport in the treated ones.

Hypothesis for Sprouting

Aguilar *et al.* (*20*) proposed the following hypothesis to explain nerve sprouting. The target tissue continually manufactures a substance that stimulates nerves to sprout, and this effect is neutralized in some way by the release of neural factors which are carried to the endings by neuronal transport. This mechanism represents a negative feedback control system to regulate the density of nerve endings at the target. This hypothesis also explains "denervation-sprouting." As a consequence of the elimination from cut nerves of the neural factors, the preexisting balance between them and the target-tissue growth-promoting substances is disturbed. The intact nerves then sprout until the new nerve terminals can release enough of the neutralizing factors to restore the original equilibrium. Consistent with this hypothesis is our finding that for every ending lost by nerve section, a new one appears from the adjacent nerves; that is, sprouting ceases when the original number of endings is restored (Fig. 1A). At least one implication of this hypothesis is that it accounts for the local acquisition of territory by nerve endings (and even individual nerves) during primary development, since each ending releases factors that hinder other endings from sprouting into its own immediate region [see (*1*)]. In the salamander skin a finding consistent with this hypothesis is that each mechanosensory nerve axon acquires an area of skin which it virtually "owns"; there is a mosaic of axonal receptive fields with only slight overlapping (*22*).

Spatial Relations and Sprouting

It is largely accepted that nerve fields are spatially organized, some overlapping, others not. If, as we have indicated, the production of terminal fields involves sprouting whose density is regulated by the mechanisms described above, then there must be some territorial control of these mechanisms. This control could be achieved by an appropriate proximity between a nerve and its prospective target tissue at the correct time during embryonic development. We further investigated our model of nerve sprouting by asking whether there are territorial controls of nerve sprouting in the mature organism. From the results we might hope to learn something of the extent to which the apportioning of territory during development is permanent, and whether it could be subject to continual remodeling during the life of the animal.

We mapped the mechanosensory field areas in the hind limbs of salamanders by brushing the skin and recording the evoked afferent impulses. The three segmental nerves that supply the limb have precisely defined fields, which, although they vary from animal to animal, are symmetrical between the two hind limbs. The 16th nerve innervates most of the dorsal surface. When this nerve is cut or treated with colchicine, the fields of nerves 15 and 17 enlarge, reaching a maximum in 8 to 12 days (Fig. 2, A and C). This enlargement, however, continues only until the two fields meet; they never overlap. In many animals the fields of nerves 15 and 17 already have a common frontier between them (Fig. 2B), and in these cases there is no area enlargement when nerve 16 is cut, although nerves 15 and 17 do sprout *within* their own fields (Fig. 1A). To investigate the possibility that a competitive situation exists here, whereby nerves actively exclude each other from their own territory, two nerves to the limb were cut, either 15 and 16, or 16 and 17.

Surprisingly, neither of the nerves 15 and 17 takes over the other's territory during the normal period when sprouting occurs, and, since the territory was totally denervated, the result could not be due to competition between the two nerves. One possible explanation, that there is a mechanical barrier to sprouting between the 15th and 17th fields (*25*), was excluded by a fortuitous finding. The 16th nerve has two branches, the anterior (16A) and posterior (16P), which join respectively the 15th and 17th nerve

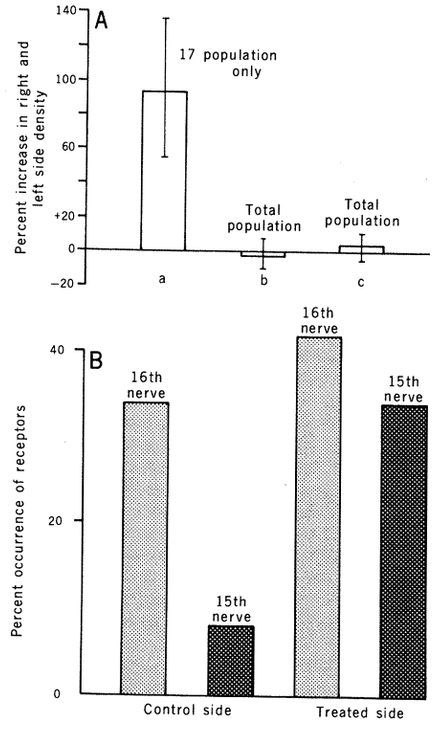

Fig. 1. (A) Quantitative sprouting after partial denervation. We measured the percent occurrence (the density) of the low-threshold touch spots (*22*) in a region of skin shared by the 16th and 17th nerves, and compared the values between right and left limbs. In the normal animal there is a high degree of symmetry between the two sides, that is, the total number of receptors is the same and so is their distribution among the spinal nerves which supply the corresponding regions of skin. Column a refers to a group of animals in which the right 16th nerve had been sectioned 3 weeks previously and shows the percent difference between right and left for the 17th nerve touch-receptor population only. Normally there is no difference. The increase in 17th nerve receptors is clearly seen. Column b shows, for the same group of animals, that there is no right-left difference when the total receptor population is compared (on the treated side there was only a 17th nerve innervation, but on the control side there was a shared 16th and 17th nerve supply). Column c shows the absence of a right-left difference in total population of touch receptors in a control group of animals, with 16th and 17th nerves intact on both sides. There is no significant difference between columns b and c; this indicates that the increase in 17th nerve receptors on the right side of the experimental group had quantitatively made up the loss due to 16th nerve section (vertical bars = S.E.M.). (B) Sprouting after colchicine treatment of adjacent nerve. Results from a single animal show touch-receptor density in a region of skin shared by the 15th and 16th nerves. In this animal the number of touch receptors associated with the 15th nerve was only a small proportion of the total. There was no loss in the population of receptors supplied by the right colchicine-treated 16th nerve, compared to the left (the increase is within the normal variation). However, on the right side the 15th nerve supplied a significantly increased population of receptors.

trunks (Fig. 2A); the frontier between the two 16 subfields is also that up to which 15 and 17 will grow. We found that, in many animals, when we cut nerves 15 plus 16A or nerves 17 plus 16P, the remaining branch of 16 invaded the denervated region beyond the frontier; however, nerves 15 and 17 stopped at it. We conclude then that there is no mechanical barrier to sprouting across the boundary region. Over significantly longer periods (1½ to 3 months), which we are examining, nerve 17 displays a clear capacity to make a limited incursion into the area of nerve 15 in the distal part of the limb although 15 has never invaded the nerve 17 area.

Another possibility to explain the apparent restriction in sprouting is that the nerves were close to an upper limit of field area that they could supply by sprouting. We excluded this by cutting all the nerves in the leg except for one 17th nerve branch, which supplied a region of skin adjacent to the boundary; extensive sprouting of this branch occurred, but only within the permitted zone—the 17th nerve field.

Location of Territorial Control

In the absence of a mechanical barrier to sprouting, of competition between the nerves, or of an inadequate capacity to enlarge their territories sufficiently, we considered the possibility that the skin stimulus to sprout is specific to each area. To test the possibility of specificity of the sprouting stimulus, skin rotation experiments were performed. Areas of hind limb skin (up to 100 mm^2) were rotated 180°, and their reinnervation patterns were studied (Fig. 3). The results were unexpected, since the ingrowing 15th nerve fibers created a new frontier, which was coincident with the position on the limb of the original 15–17 one, as though no skin rotation had occurred at all, and showed no preference for their original skin. This result was not simply dependent on the presence and location of the surviving central nerve stumps beneath the rotated skin flap. In some experiments in which the limb was partially denervated in addition to rotation of the skin (for example, by section of nerves 17 and 16P), the uncut branch of the 16th nerve (16A) was observed to grow across the rotated implant, as it often does in normal unrotated skin. However, the 15th nerve in most animals was confined to the newly created frontier even when this frontier straddled a region that was originally part of the 15th nerve's territory (Fig. 3). Nerve 17 tended to spread more or less indiscriminately over the transplant along with the corresponding (posterior) branch of the 16th nerve. Probably all of the fibers that invaded the transplant were those cut at the border of the excised piece of skin, and thus were regenerating. We conclude that the tendency for nerve 17 to "break out" of its territory, normally requiring both a longer time to be expressed and appearing primarily in the distal part of the limb, is enhanced when the nerve is regenerating. This is in contrast to the more rigorous containment of nerve 15 within its territory. Differences between regenerating and sprouting intact nerves are discussed below.

In summary, nerve 15 refuses to sprout into skin normally supplied by nerve 17 when that skin is in situ, and yet invades it when it is transplanted into the original territory of nerve 15, but only up to the line that defines the limit of that territory on the surface of the limb. Moreover, the 15th nerve is apparently unable to invade even a sector of its own skin, which occupies a position beyond that line. The 17th nerve initially is governed by similar territorial limitations. It becomes less so in the distal part of the limb with the passage of time, or when it is regenerating. Finally, nerves 16A and 16P are able to cross the frontier more readily than either nerve 15 or 17.

In interpreting the above findings, we must first abandon *skin* fields as defining nerve territory and instead substitute a territory defined with reference to some coordinate system, which must relate to the body. We refer to this coordinate system as "body space." For the 15th nerve especially, the body space rigorously determines where the nerve will sprout, that is, where its nerve field occurs. Second, nerves seem capable of sprouting functional endings nonselectively, even into "foreign" skin, provided that skin is within the body-space territory of the nerve. Third, and important in its implications, the stringency with which the nerve field is governed by or complies with this body-space territorial limitation, is variable. There may be a time dependency, in that the intact 17th nerve conformed to the body-space control only for about 1 month, and the 15th nerve for as long as we have followed it (at least 4 months). Finally, our results indicate that nerves which do "break out" of their body space to invade adjacent denervated skin do so more readily in the distal, rather than proximal, region of the limb.

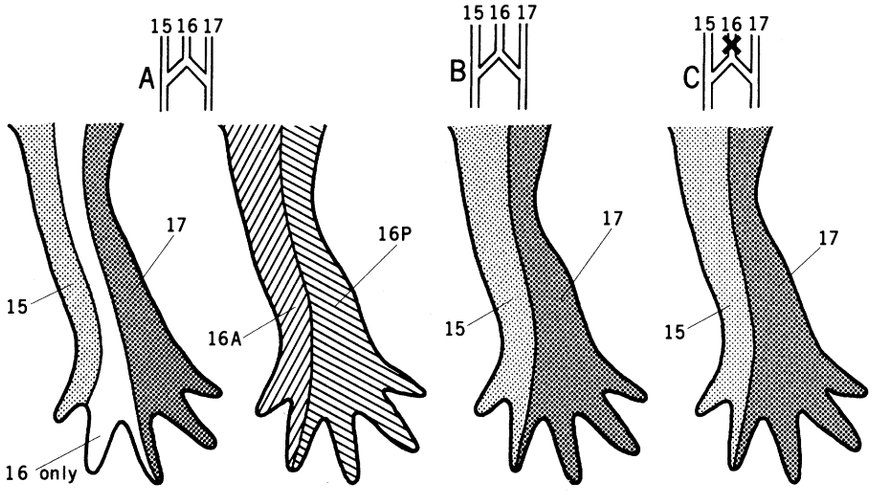

Fig. 2. Nerve territories in dorsal skin of salamander hind limb. (A) Control limb in which fields of nerves 15 and 17 do not meet. The two subfields of nerve 16, 16A and 16P, are shown separately for clarity; the whole of this skin was supplied by the 16th nerve, which overlapped with the other two nerves as shown. (B) Control limb in which fields of nerves 15 and 17 meet. (C) Experimental limb in which nerve 16 has been cut, showing enlargement of the fields of nerves 15 and 17, which, in the control limb, were as shown in (A); they now meet at the common border between 16A and 16P. The fields of nerves 15 and 17 of a limb similarly treated, whose fields are shown in (B), were unchanged in area, although the *density* of their endings increased (see text). For convenience, in this and subsequent figures the control and experimental limbs are represented with the same anteroposterior orientation.

Sprouting of Regenerating Nerves

According to our hypothesis for nerve sprouting, the prior occupancy of skin by a nerve will have neutralized the sprouting stimulus. Therefore, under normal conditions there is no tendency for invasion by adjacent nerves. However, such invasion may occur when the occupying nerve is cut, or when its neuronally transported factors are reduced by

colchicine. Invasion by adjacent nerves, however, depends on how rigorously the body-space limitations are imposed and possibly on how long the now excessive skin stimulus is permitted to be available. Regenerating nerves, in contrast, appear to have special qualities. When we cut all three nerves and redirected the regenerating fibers of nerve 15 into the distal stump of the cut 16th nerve (while preventing regeneration of 16 and 17), we found that they were apparently guided to "foreign" skin and formed mechanosensory endings in it which functioned quite normally. A similar finding has recently been reported (*26*). Moreover, the redirected 15th nerve fibers showed no apparent preference for the 16A branch (which would have guided them to their original territory) but freely entered both divisions of nerve 16.

In the example shown (Fig. 4, A and B), the innervation pattern was relatively uncommon in that the posterior branch of the 16th nerve failed to supply the most lateral strip of limb skin, which therefore received only 17th nerve fibers. The regenerating 15th nerve fibers filled both 16A and 16P fields, although the latter overlapped with the field of nerve 17. However, they did not invade the strip previously occupied by nerve 17 only. It seems, therefore, that, outside the limits of the presumed mechanical guidance provided by the degenerating trunk of nerve 16, the body-space territorial limitation applied. While they are actively regenerating, therefore, nerves may be guided into normally alien territory (see *27*).

Simultaneous collateral sprouting and regeneration. A nice example that distinguishes between the territorial containment of the sprouting of intact nerves, in contrast to that of regenerating ones, was obtained in the following kind of experiment. The 15th and 16th dorsal root ganglia were removed, and all the nerves to the limb were cut except for one branch of the 17th. This branch sprouted up to the border between the 15th and 17th territories, and stopped there. However, the skin field of nerve 15 did eventually become completely innervated by the regenerating fibers from the other cut branches of the 17th nerve; these fibers had grown up, around, and into the denervated stump of 16A, which guided them downward into the skin territory of 16A and 15 (*28*).

There is another reason for believing that there are differences between the sprouting of intact and of regenerating nerves. In winter we found that salamander nerves frequently will not sprout collaterals at all after the cutting of adjacent nerves, although the distal portions of the cut nerves always underwent the usual Wallerian degeneration. However, the cut nerves always regenerate quite normally to produce new functional endings in skin and muscle. It seems that regenerating nerves are not under the control of the sprouting stimulus from the target tissue, but more likely respond to some innate "drive" that is triggered in an all-or-nothing fashion by nerve section; given mechanical guidance they are not specially sensitive to the foreignness of the body region invaded. Our findings with regenerating nerves suggest that they may not always provide an appropriate model of the development of normal innervation (see *29*). In contrast to regenerating nerves, normal intact nerves sprout in a graded manner, dependent on the balance between the peripheral stimulus (which may be reduced in salamanders during winter) and the neuronally transported factors; they are also to a greater or lesser extent governed by limitations imposed by their body space (see above).

We also investigated the question of whether nerves regenerating along with a limb newly growing after complete amputation in the adult animal would form normal fields. When one spinal nerve was given an opportunity to innervate the blastema in advance of the other two (*30*), all three spinal nerves overlapped everywhere in the limb (Fig. 4D). However, when all three nerves were allowed to regenerate simultaneously, there was some restoration of the normal pattern of innervation. We are further investigating the extent to which territorial limitations become expressed in this type of situation.

Discussion

As to general conclusions that may be made from these results, of most importance is the indication that there exists throughout life a means of continuous regulation of nerve fields. This regulation involves an interaction between the effects of "sprouting-stimuli" produced in or near the target tissue, and of factors brought to the periphery by neuronal transport. During development there is evidence that an initial "overshoot" in sprouting occurs at muscle, followed by a regression of endings to achieve an appropriate pattern of innervation (*31*). An ideal control system should be able both to increase and to decrease the density of nerve endings. The mechanism that regulates sprouting, however, is subject to limitations imposed on the nerves by their occupancy of territories in body space. These determine the extent to which nerves will or will not enlarge the areas of their terminal fields. The ability of nerves to "break out" of their territories varies, possibly, with the position of the origin of the nerves along the neuraxis, with the time over which they are exposed to the sprouting stimulus, and with the distance from the central axis of the body. The establishment of distinct body territories occurs presumably during primary development by as yet unknown mechanisms and could involve chemical gradients (*32, 33*). Perhaps the territories we have suggested as being defined in body space are analogous to the compartments discovered in *Drosophila* (*34*), whose possible role in controlling shape and size was discussed by Crick and Lawrence (*35*). The imposing of spatial character on a nerve fiber population could require the participation of the peri-

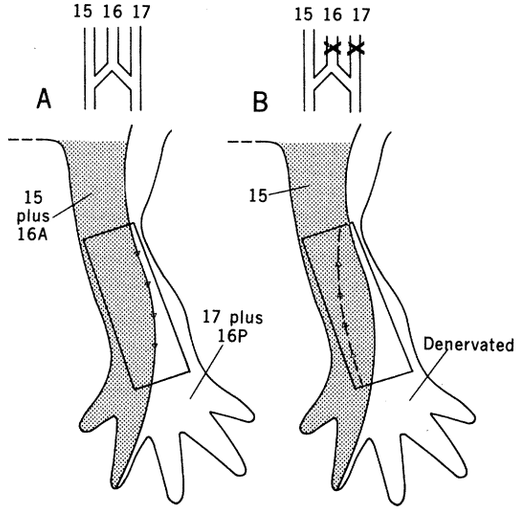

Fig. 3. Effects of rotating skin areas on nerve fields. (A) and (B) refer to an experiment in which the rectangle of skin shown in limb (A) was rotated 180° in limb (B). The unshaded area in the control limb (A) is the field of nerves 16P and 17. The 15th field is shaded. In the experimental limb both the 16th and 17th nerves had been cut at the time of skin rotation. The unshaded area shows the denervated region of skin. The dashed line in the skin rectangle of limb (B) shows the original boundary between the 15th and 17th fields in the skin before rotation. (This was also the boundary between fields of 16A and 16P; see Fig. 2.) After sprouting and invasion of the rotated skin flap, the new 15 boundary established in limb (B) skin is in the identical position in body space to that in the control limb (A).

neurium or some analogous structural component in the CNS; such an involvement of nonneural cells may confer on these a role well beyond that of metabolic or mechanical support.

Our findings are of interest in relation to the results of other experiments which suggest that skin can, at an early enough stage of development, apparently provide information about its location in the body to the incoming nerve, which is then used to construct appropriate reflex circuitry in the spinal cord (*36*). Possibly the body space influence on the nerve we describe is that which confers regional specificity on the skin too. If so, then the spatial influence, while it directly controls the areas of nerve fields at the level of the peripheral target, may affect the central connections of the nerve mostly by this indirect means. In the experiments quoted above, if respecification of rotated skin occurred, it took too long a time for its result to be effective in the CNS. What is clear is that a regional character of skin does not affect the peripheral fields of the incoming nerves (*36*), but only their central reflex connections.

The mechanisms involved in the interactions between neuron and target, along with the existence of spatial influences on sprouting, help us to understand a variety of related phenomena involving the terminal nerve sprouting of normal nerves as well as that of denervation sprouting. The sprouting found by Olsen and Malmfors (*17*) into the iris transplant in the anterior chamber of the eye clearly indicates a target stimulus. The sprouting of motor nerves treated with botulinum toxin (*19*) would also be explained if, in addition to release of acetylcholine, release of the postulated neural factors was also blocked by the toxin. Another phenomenon that becomes understandable is the way in which the density of endings apparently remains constant during the growth of an organ or tissues (*3*), suggesting that a peripheral stimulus is involved in the initiation of sprouting (*1, 18, 37*).

Neural mechanisms of the sort we propose should have their counterparts in the CNS; there is evidence, for example, that spatial gradients may control nerve fields in the visual system (*33, 38*). Hubel, Wiesel, and Le Vay found that the cortical projections of the lateral geniculate neurons connected to one eye enlarged their territories in layer IV at the expense of adjacent territories associated with the other eye, after monocular deprivation earlier in life either by eye removal (*39*) or by lid suture (*40*). These changes presumably reflect a sprouting of one set of terminals and very likely either an arrest of growth or a regression (or both) of the other set; such changes could be related to a reduced neuronal transport in the deprived axons (see *41*). There is evidence (*20*) that axons treated with colchicine are not themselves able to respond to the sprouting stimulus, as if this ability too were dependent on neuronal transport. In any event, our hypothesis explains why a failure of one neural input to establish or maintain its normal territory could result in an unusual enlargement of the territory of an adjacent input, provided the latter's exclusion by body space territorial limitations is not absolute.

Our speculations do not exclude the possibility either of the operation of specific target influences that can guide nerves to make preferential connections (*37*), or of the establishment of territorial limitations which apply over circumscribed three-dimensional spaces such as a segment of spinal cord, a limb, or even a single neuron (*42*). What we are emphasizing is the potential importance in the mature nervous system of the dynamic regulation of the density of terminal fields, whose areas are also subject to territorial limitations. While the skin is a simple model there is no reason why the principle should not apply to any target. We now have some evidence that the influence of body space in governing nerve fields may be an attribute also of the muscle fields of the nerves we have investigated, in addition to those in the skin. Presumably, synaptic fields on neurons represent an analogous system.

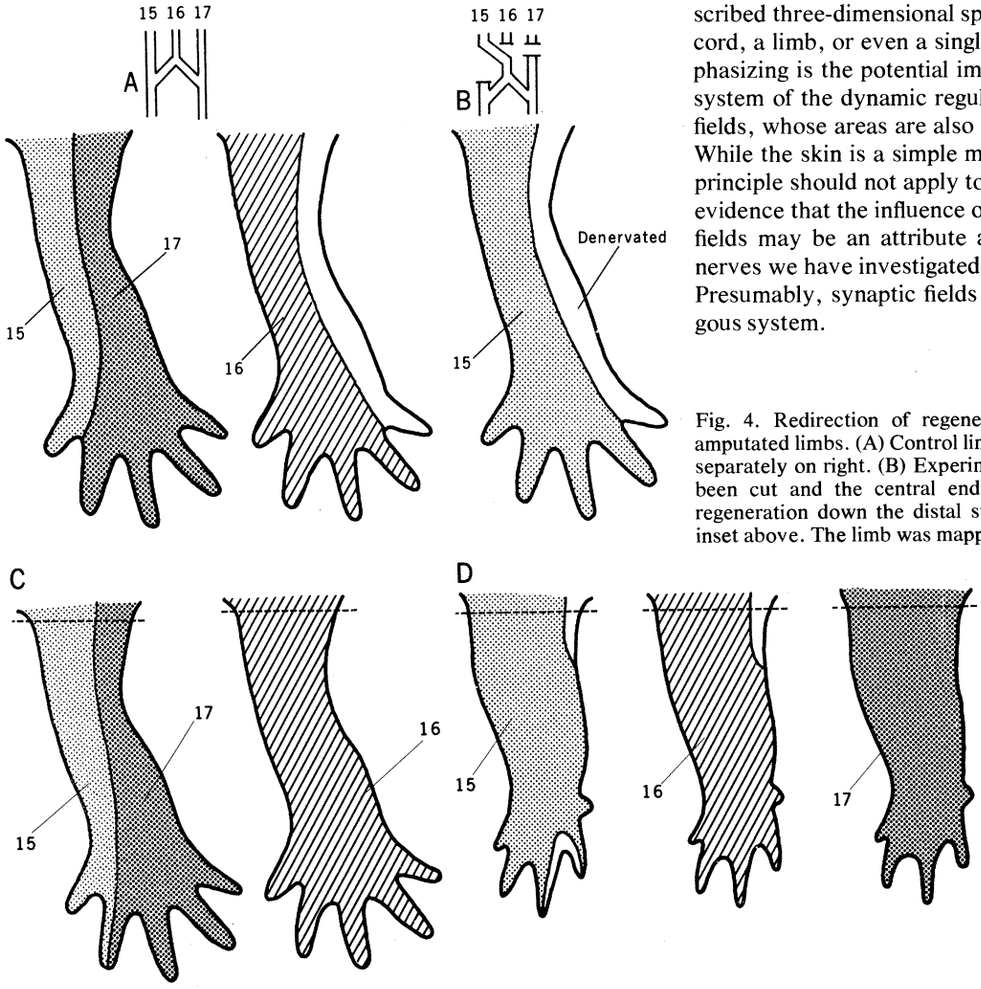

Fig. 4. Redirection of regenerating nerves and regeneration of amputated limbs. (A) Control limb with small 16 nerve field is shown separately on right. (B) Experimental limb in which all nerves have been cut and the central end of nerve 15 redirected to permit regeneration down the distal stump of nerve 16, as shown in the inset above. The limb was mapped 10 weeks later, and only the 15th nerve fibers were present. The region originally supplied by nerve 17 only was not invaded by the regenerated 15 nerve fibers. (C) and (D) Compare the 15, 16, and 17 nerve fields respectively in a control limb and in a limb which had regenerated for 4 months after amputation at the level shown by the horizontal dashed line. For clarity the 16th field is shown separately in the control, and all three fields are shown separately in the regenerate. In this experiment the 17th nerve regenerated ahead of the other two (*30*). Note that except for two very small regions, all three nerves overlapped in the regenerated limb.

Conclusion

1) Our hypothesis, that the density of endings in a given nerve field is regulated by the interaction of sprouting factors continually manufactured by the target tissue, with neutralizing (antisprouting) agents carried to the nerve endings by neuronal transport, has been strengthened by quantitative studies on the mechanosensory innervation of salamander skin.

2) After we partially interrupted fast axoplasmic transport in one nerve by colchicine application, its terminal field was invaded by sprouting fibers from neighboring axons, even though its own endings might be unchanged in number, distribution, and sensory threshold.

3) This mechanism to regulate sprouting is observed in the mature animal. Its operation, involving either provision of extra sprouting factor or reduced neuronal transport, can explain various developmental and experimental situations in peripheral and central nervous systems (such as partial denervation), in which collateral nerve sprouting occurs (*43*).

4) The area of a given nerve field (the extent of target territory over which the nerve endings occur) is susceptible to a spatial control that is not located in the target itself, but relates to the coordinate system provided by the body. Nerves will normally sprout only within their "body space" territory, which is presumably allotted to them during primary development. Some cutaneous mechanosensory nerves of the salamander, for example, will invade apparently "foreign" skin only if it is relocated in their appropriate body space, and will not usually sprout even into their own original skin if the skin lies beyond the defined boundary of their territory. This boundary however is not a mechanical barrier to sprouting. Other nerves, exposed long enough to the sprouting stimulus provided by adjacent denervated skin, can break out of their body space territory to a limited extent, especially when these particular nerves are regenerating.

5) The stimulus that initiates regeneration in a cut nerve is different from that causing collateral sprouting of intact nerves at a target tissue. Regenerating fibers seem to have "central drive" and can ignore the limitations imposed by the body space control of territory. When guided by degenerating nerve trunks, regenerating axons of a nerve will readily innervate alien territory whose border is not crossed by the collateral sprouts of intact fibers of the same nerve. In newly regenerating salamander limbs, nerves are less rigorously governed by this spatial control of territory.

6) It seems that both mechanisms, the local neuron-target interaction that regulates the density of endings within a field, and the more generalized body space control that serves to limit the permitted area of the terminal field, could conceivably be involved in adjustments of neural circuitry throughout the life of the organism.

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28. The regenerating 17th fibers that grew up and around to join the 16th nerve trunk originated from the 17th nerve trunk. We recorded the total mechanosensory 17th nerve input from that trunk. The whole dorsal skin was found to be innervated by the 17th nerve fibers. When the 16A branch was recut, all the innervation from the 16A and 15th fields disappeared, leaving only the unchanged mechanosensory input from the 17th nerve field. Thus, the 16A trunk was the route taken by the 17th nerve axons which supplied the "alien" 16A and 15th nerve field. It was then necessary to test whether these were regenerating 17th nerve fibers of the cut branches or aberrant sprouts from the uncut ones of the intact 17th nerve branch. This was done by an occlusion experiment. We electrically stimulated the 16A trunk and the intact 17th nerve branch, and recorded the compound action potentials (CAP) from the main 17th nerve trunk. Simultaneous stimulation through both pairs of electrodes gave a CAP which exactly equaled the sum of the individual CAP's obtained from stimulation of each pair of electrodes alone. This result meant that the fibers of the uncut 17th nerve branch were not responsible for branches that had grown up and around into the 16th nerve trunk.
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43. There are other possibilities than those specifically outlined in our hypothesis of nerve sprouting which our evidence does not preclude. For example, neuronally transported materials may act intracellularly to limit uptake of the sprouting factors into the nerve endings. Alternatively, the target tissue factors may be continually taken up and retrogradely transported to act at the cell body (*18*). There is a similarity between our hypothesis and the first of these two alternatives in that both involve two agents, one produced by the target tissue, the other by the nerve. Our hypothesis, however, proposes that the neural factor is released from the nerve endings. The second alternative involves only a target factor. However, it does require the assumptions that the retrograde transport of this factor is blocked by colchicine, and that this leads to a damming up of the factor sufficiently to limit its uptake at the nerve endings and so cause a buildup in the target tissue.

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