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A CONDITIONING LESION OF THE PERIPHERAL AXONS OF DORSAL ROOT GANGLION CELLS ACCELERATES REGENERATION OF ONLY THEIR PERIPHERAL AXONS[1]

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Abstract

Axotomy of the peripheral axon of dorsal root ganglion (DRG) cells is known to result in chromatolysis and changes in protein synthesis in DRG cells. We investigated whether a stimulus produced by peripheral branch axotomy would affect the regenerative properties of both the central and peripheral axon of the DRG cell equally. To examine this question, a conditioning crush lesion was made distally on the sciatic nerve 2 weeks prior to a testing lesion of either the dorsal root or peripheral branch axon near the DRG. Fast axonal transport of radioactive proteins was used to assess regeneration of DRG axons. In the adult rat, leading peripheral branch axons normally regenerate at a rate of 4.4 mm/day. If a conditioning lesion of the sciatic nerve is made 2 weeks before the test lesion, the rate of peripheral branch axonal regeneration increases by 25% to 5.5 mm/day. This effect is not limited to the fastest growing axons in the nerve since a population of more slowly growing axons also exhibits accelerated outgrowth in response to a prior peripheral axotomy. In contrast to this, the fastest growing central branch axons of DRG cells, which normally regenerate at a rate of 2.5 mm/day, are not significantly affected by a prior peripheral axotomy. A population of more slowly growing axons in the dorsal root also does not exhibit accelerated outgrowth in response to a peripheral conditioning lesion.

The results of these experiments indicate that changes in the DRG neuron's metabolism induced by prior axotomy of its peripheral axon do not affect the regenerative properties of both axons equally. This raises the possibility that accelerated axonal outgrowth in only one axonal branch results from a differentially regulated supply of proteins to the two axons by the DRG cell body.

The peripheral extensions of neurons, such as dendrites and axons, are readily distinguished by structural, functional, and biochemical criteria. The selective routing of materials from the cell body into particular neurites may play a central role in establishing such regional differences in different parts of a neuron. Studies of axonal transport in dorsal root ganglion (DRG) cells have provided support for this suggestion. The DRG cell is well suited to the study of differential routing since it has two long, functionally distinct axons that branch from a short stem axon. One branch courses centrally through the dorsal root to synapse on central neurons, and the other proceeds peripherally to terminate on sensory receptors. A number of asymmetries in the supply of proteins from the DRG cell body to the two axons exist. For example, the amount of rapidly transported protein (Lasek, 1968; Ochs, 1972; Ochs et al., 1978), the amount of cytoskeletal protein carried in slow axonal transport, and the rate of slow transport are greater in the peripheral axon than in the central axon (Lasek, 1968; Komiya and Kurokawa, 1978; Mori et al., 1979; Lasek et al., 1983; Wujek and Lasek, 1983).

Differences in axonal transport are of importance because they may determine several different structural and functional features of the peripheral and central DRG axon. For example, the diameter of the peripheral axon is larger than that of the central axon (Suh et al., 1984). This structural difference, in large part, results from the differential amounts of slowly transported cytoskeletal polymers entering the two branches and the differential rates of transport of these elements in each axonal branch (Lasek et al., 1983). Axonal transport of the cytoskeleton is clearly involved in axonal regenera-

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tion and may determine the rate of axonal elongation (Lasek, 1981; Lasek and Hoffman, 1976; Lasek et al., 1981; McQuarrie, 1983). A notable functional difference between the two axonal branches of the DRG cell is that the rate of regeneration of peripheral branch axons is about twice that of dorsal root axons (Komiya, 1981; Wujek and Lasek, 1983). The axonal regeneration rate correlates with the rate of slow transport, particularly of slow component *b* (SCb), in each axonal branch (Komiya and Kurokawa, 1978; Komiya, 1981; Wujek and Lasek, 1983). These observations indicate that a differential supply of proteins to the two branches of the DRG cell may affect the functional properties of these axons.

An interesting question arises when considering the maintenance of two quite distinct axons by the DRG cell body: Would a stimulus which alters the metabolism of the neuron change the regenerative properties of its two axons equally? In this paper we address this question by stimulating a chromatolytic response in the DRG cell in order to produce changes in protein synthesis in the cell body. Chromatolysis can be induced in mammalian DRG cells by cutting the peripheral, but not the central, axon (reviewed in Cragg, 1970; Lieberman, 1971). This results in changes in the levels of cytoskeletal protein synthesis (Perry and Wilson, 1981; Hall, 1982). The effects of these cell body changes on the peripheral and central axons were assessed by measuring the regenerative capacity of the axons 2 weeks after inducing the chromatolytic response. This experimental approach, a conditioning lesion paradigm, which entails inducing a neuron to regenerate with one lesion and testing the effects of this lesion with a subsequent axotomy, is known to result in an acceleration of the rate of axonal regeneration in several systems. (McQuarrie and Grafstein, 1973, 1981; McQuarrie et al., 1977; Grafstein and McQuarrie, 1978; Forman et al., 1980, 1981; McQuarrie, 1981, 1984). The effects of a conditioning lesion on subsequent axonal regeneration have been related to changes in the synthesis and export of proteins from the cell body into the axon. For example, changes in the synthesis and transport of slowly transported cytoskeletal proteins have been correlated with increases in the rate of axonal elongation (reviewed in Grafstein and McQuarrie, 1978; Forman et al., 1981; Lasek et al., 1981; McQuarrie and Grafstein, 1982; McQuarrie, 1984).

We report that a peripheral conditioning lesion increases the rate of axonal elongation of only peripheral axons of DRG cells. This observation suggests that changes in protein synthesis during the chromatolytic response to axotomy are differentially transferred to the two axons of the DRG cell, and it provides support for the hypothesis that the cytoskeletal networks in different regions of the neuron can be selectively regulated.

Materials and Methods

Test lesion. The experiments utilized young adult male Sprague-Dawley rats (200 to 300 gm). For all surgical procedures, animals were anesthetized with a mixture of sodium pentobarbital (27 mg/kg) and chloral hydrate (128 mg/kg). Regeneration of DRG axons was assessed after an axotomizing test lesion. The test lesion consisted of crushing either the fifth lumbar (L5) spinal nerve or the L5 dorsal root at 8 to 10 mm from the L5 DRG (Fig. 1). The L5 spinal nerve was exposed at the L4–L5 spinal nerve junction outside of the vertebral column; the L5 dorsal root was exposed at the level of the L4 DRG by a partial laminectomy. The lesion consisted of crushing the nerve twice with a #5 Dumont jeweler's forceps for 15 sec, a method that completely transects all axons of the nerve (McQuarrie et al., 1977). At the end of surgery, overlying muscles, fascia, and skin were closed with silk suture.

Conditioning lesion. Some animals received a conditioning lesion 2 weeks prior to the test lesion (Fig. 1). The conditioning lesion consisted of crushing the sciatic nerve in the midthigh (50 to 60 mm from the L5 DRG) with #5 Dumont jeweler's forceps twice for 15 sec. Two weeks later, a test lesion was made as described above. A 2-week conditioning interval was used since this interval has been shown to be optimal for various neuronal systems studied to date (Grafstein and McQuarrie, 1978; Forman et al., 1981).

This paradigm provided four groups of animals: one group had only a test crush of the peripheral branch of the DRG (spinal nerve); one group had only a test crush of the central branch (dorsal root); a third group sustained a conditioning lesion of the peripheral branch (sciatic nerve) 2 weeks prior to a test crush of the peripheral branch (spinal nerve); and a fourth group sustained a conditioning lesion of the sciatic nerve 2 weeks prior to a test crush of the dorsal root (Fig. 1).

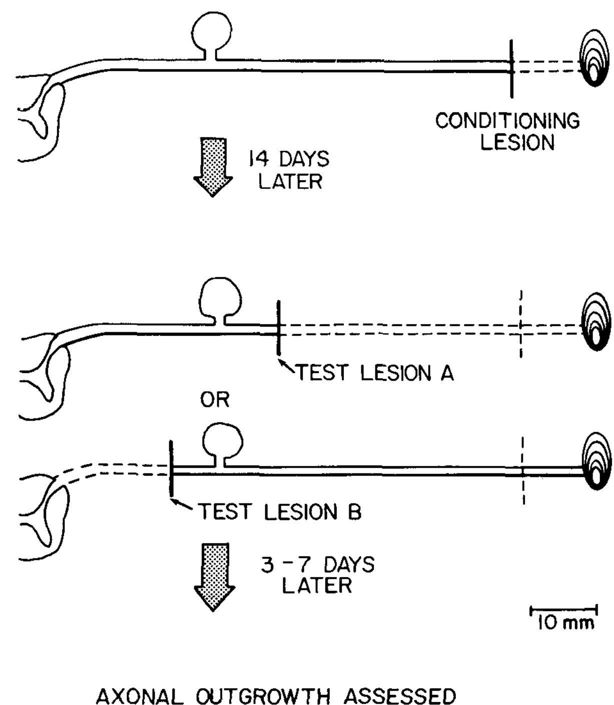

Figure 1. Schematic showing sites of lesions in the experiments. The conditioning lesion was a crush of the sciatic nerve in the midthigh (50 to 60 mm from the L5 DRG). Test lesions were done either alone or 2 weeks after a conditioning lesion. Test lesions were crushes of either the L5 spinal nerve (test lesion A) or the L5 dorsal root (test lesion B) at 8 to 10 mm from the DRG.

Measurement of axonal regeneration. Survival intervals of 3 to 7 days after test lesions were used to allow axonal regeneration to progress. Regeneration was measured by labeling the regenerating axons via fast axonal transport of radioactive proteins (Forman and Berenberg, 1978). At 18 hr prior to sacrifice, the L5 DRG was exposed by a partial laminectomy, and a glass micropipette was used to inject a 1:1 mixture of [^{3}H]proline and [^{3}H]lysine (New England Nuclear) that was concentrated to 100 μCi/μl. The injection volume of 1.0 μl was delivered to the midpoint of the L5 DRG at a rate of 0.1 μl/min. The wound was closed after injection, and the animals returned to their home cages for 18 hr. After that interval, rats were sacrificed by decapitation, and the sciatic nerve, L5 DRG, and L5 dorsal root were rapidly removed and frozen.

The entire L5 nerve system was cut into consecutive 1-mm segments. The segments were solubilized in 250 μl of Soluene-350 Tissue Solubilizer (Packard) at 60°C overnight. A 5-ml volume of scintillation cocktail (3 gm of 2,5-diphenyloxazole, 12 gm of benzoic acid in 1 liter of toluene) was added to each sample, and the radioactivity was determined using a Beckman LS-335 liquid scintillation counter.

Two methods of assessing axonal regeneration were employed. The first provided information on the rate of regeneration of the fastest growing axons. Regeneration distances were measured at different postcrush survival times. To determine the regeneration distance, the radioactivity of each 1-mm segment was plotted as a function of distance from the DRG. The radioactivity determinations (counts per minute) were corrected for tissue background. Tissue background for each experiment was determined by averaging the radioactivity in very distal segments of crushed nerve where no axons had reached. This low level background radioactivity is due to bloodborne labeling of non-neural tissue (Forman and Berenberg, 1978). The furthest distance from the crush site at which the radioactivity level was at least 2 SD above mean tissue background radioactivity defined the outgrowth distance of the fastest growing axons. Axonal regeneration rates were determined by the slope of the linear regression function of regeneration distance on survival time. The regression method of obtaining regeneration rate has been extensively used in both sensory and motor systems, where it has been established that the rate of axonal elongation is linear (Gutmann et al., 1942; McQuarrie et al., 1977; Forman and Berenberg, 1978; Black and Lasek, 1979; Forman et al., 1980; Pestronk et al., 1980; Wujek and Lasek, 1983). By extrapolating the linear regression function to zero outgrowth distance, an estimate of the initial delay was obtained. This initial delay represents the time required for axonal sprouting from the proximal axon and for traversing both any proximal zone of traumatic degeneration that exists and the crush site.

The second method used to assess regeneration provided information on the entire population of regenerating axons that had traversed the crush site at various postcrush times. The total radioactivity of nerve segments distal to the crush site was calculated. The percentage of this total contained in each 1-mm segment of nerve distal to the crush was calculated. The radioactivity in these segments is contained primarily in growth cones with some label being present in axon shafts (Griffin et al., 1976; Bisby, 1978, 1979; Forman and Berenberg, 1978; McQuarrie, 1981). This method essentially provides a way of normalizing data from animals in the same experimental group for differences in overall radioactivity levels that are due to factors such as variations inherent in injection procedures, and it allows averaging of data from groups of similarly treated animals.

Results

Comparison of normal regeneration in two axonal branches of DRG cells. The radioisotopic labeling method provides a sensitive assay of regeneration of axons in the two branches of the DRG cell. The typical pattern of radioactivity obtained using fast axonal transport consisted of very high levels of radioactivity in regions of the nerve proximal to the crush; distal to the crush site high levels of radioactivity decreased to reach a constant low level of background labeling. Figure 2 depicts profiles

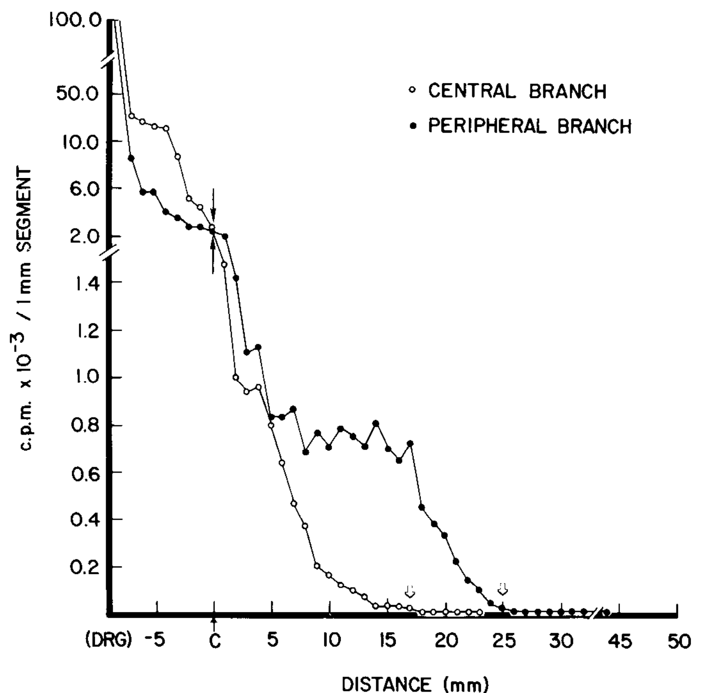

Figure 2. Distribution of fast axonally transported radioactivity in regenerating L5 sensory axons. At 7 days after a test crush, the radioactivity profiles of a peripheral branch (*solid circles*) and a central branch (*open circles*) of the DRG from two representative experiments are depicted. The radioactivity (counts per minute, corrected for background) of each 1-mm segment of nerve is plotted as a function of distance from the test crush (zero distance). The location of the test crush in both experiments is indicated by *large solid arrows.* The *open arrows* point to the leading edge of the fastest growing axons in both cases. These distances represent the furthest points at which the radioactivity levels were greater than 2 SD above the stable low level tissue background labeling in distal nerve segments. These two examples are representative of the consistent finding that axons of the peripheral branch extended further from the crush site than axons of the dorsal root at identical crush-sacrifice intervals.

from two experimental animals at 7 days after a test crush of either the dorsal root or the peripheral sensory branch. This example serves to illustrate the results typically obtained at various postcrush intervals: The outgrowth distance of leading axons in the peripheral branch was greater than that of leading axons in the central branch (*open arrows*, Fig. 2). At both 5 and 7 days after a test crush, the mean outgrowth distance of leading axons of the peripheral branch was significantly greater ($p < 0.05$) than that found in the central branch (compare Figs. 3 and 4).

The rate of elongation of sensory axons is known to be linear (for example, see McQuarrie et al., 1977; Forman et al., 1980; Wujek and Lasek, 1983). Our data were consistent with these observations because the correlation coefficients of the linear regression functions of outgrowth distance on time for both the peripheral branch ($r = 0.99$) and the central branch ($r = 0.96$) axons in the groups receiving a test lesion alone (Figs. 3 and 4) are significant ($p < 0.05$). The slopes of the regression lines provided the rates of regeneration of the fastest growing axons. The rate of regeneration of the leading peripheral sensory axons after a single test crush was 4.4 mm/day (Fig. 3). This value is similar to previously reported rates of regeneration of leading sensory axons obtained by various methods of ascertaining outgrowth in the rat sciatic nerve (McQuarrie et al., 1977; Bisby, 1978, 1979; Pestronk et al., 1980; Wujek and Lasek, 1983). The rate of regeneration of leading dorsal root axons was 2.5 mm/day (Fig. 4). This rate is similar to that found in a recent study of dorsal root regeneration in the rat (Wujek and Lasek, 1983). The difference in regeneration rate of leading peripheral and central branch axons is significant (difference in slope, $p < 0.05$; Armitage, 1974).

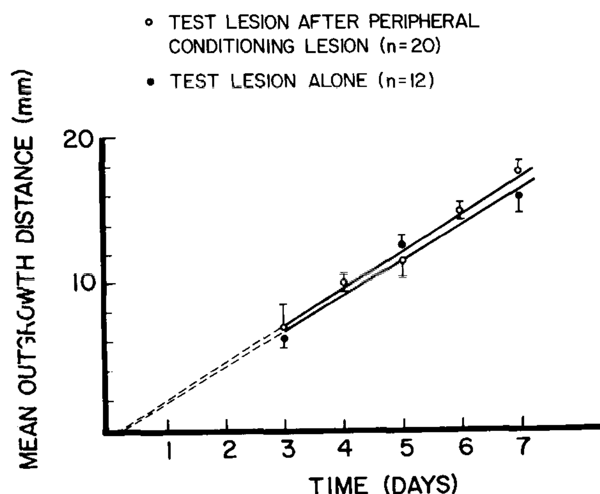

Figure 4. Effects of a peripheral conditioning lesion on regeneration of leading axons in the central branch of the DRG. Mean outgrowth distances (±SEM) are plotted as a function of time after test lesions. Regression lines were fit to the data of each group by the method of least squares. The rates of regeneration obtained from slopes of the linear regression functions are 2.5 mm/day for the test lesion alone group and 2.6 mm/day for the group which received a prior conditioning lesion of the sciatic nerve. Extrapolations of the regression lines to zero outgrowth distance (*dashed lines*) provide estimates of the initial delays.

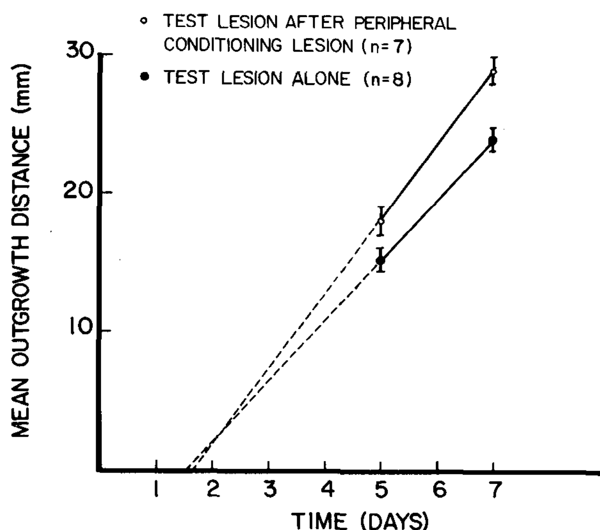

Figure 3. Effect of a peripheral conditioning lesion on regeneration of leading axons in the peripheral branch of the DRG. Mean outgrowth distances (±SEM) are plotted as a function of time after test lesions. Regression lines were fit to the data from the two groups of animals by the method of least squares. The rates of regeneration obtained from the slopes of the regression lines are 4.4 mm/day for the test lesion alone group and 5.5 mm/day for the conditioning lesion group. Extrapolations to zero outgrowth distance (*dashed lines*) provide estimates of the initial delays.

Because our data, as well as many previous reports, indicate that the rate of axonal elongation is linear, an estimate of initial delay can be obtained by extrapolation of the linear regression function of outgrowth distance on time to zero distance. For leading axons of the peripheral branch (test lesion only group), the initial delay was estimated to be 1.5 days (Fig. 3), consistent with previous observations (McQuarrie et al., 1977, Bisby, 1978, 1979; Wujek and Lasek, 1983). The initial delay prior to elongation of leading dorsal root axons after a single test crush was 0.4 day (Fig. 4; see also Wujek and Lasek, 1983). This method of obtaining initial delay does not provide a rigorous way of assessing the time required for initial sprout formation but, instead, gives an estimate of the total time elapsed before new axons are detected in the distal nerve. Therefore, it includes the time required for initial sprouting and the time used in traversing any proximal zones of degeneration as well as the crush site.

Fast axonal transport of labeled proteins appears preferentially to label the distal ends of regenerating axons (Griffin et al., 1976; Forman and Berenberg, 1978). Therefore, this method provides information on the distribution of the entire population of regenerating axons that have entered the distal nerve. Clearly, not all of these axons elongate at the rate of the leading axons (Fig. 2). At various times after a test crush, greater levels of radioactivity were distributed further distal from the crush site in the peripheral branch than the central branch (Fig. 2). This pattern could be more clearly seen when data from control nerves (test lesion only groups) at 5 days postcrush were compared by plotting the aver-

age percentage of total postcrush radioactivity contained in each segment of nerve distal to the crush (compare Figs. 5 and 6). Greater proportions of radioactivity extended further from the crush site in the peripheral branch than in the central branch (for example, compare 5% level, Figs. 5 and 6, control data). This indicated that a larger number of more slowly growing axons in the peripheral branch had progressed further from the crush site than such axons in the central branch. Also, in the peripheral branch, there was a peak of radioactivity indicative of larger numbers of growth cones (Fig. 5, control data). However, in the dorsal root, no such peak was evident (Fig. 6, control data). This suggests that growth cones of more slowly advancing axons were more widely staggered from the crush site in the dorsal root compared to the peripheral branch at that time.

Effect of a conditioning lesion on regeneration of leading axons in the two branches of DRG cells. A peripheral conditioning lesion made on the sciatic nerve 2 weeks prior to a test crush of the peripheral branch accelerated the outgrowth of leading axons in the peripheral branch. At both 5 and 7 days after a test crush, the mean outgrowth distance of leading peripheral sensory axons was significantly greater ($p < 0.05$) in the conditioning lesion group than in the control group (Fig. 3). In both groups, the axonal outgrowth distance increased linearly with time after the testing lesion (Fig. 3). The rate of regeneration of the leading peripheral sensory axons in the conditioning lesion group was 5.5 mm/day; in control axons, this rate was 4.4 mm/day (Fig. 3). The difference in regeneration rate of leading axons in the two groups is significant (difference in slope, $p < 0.05$; Armitage, 1974). While a 25% increase in the elongation rate of leading axons resulted from a conditioning lesion, there appeared to be no change in the initial delay (Fig. 3). The initial delays for the conditioned and unconditioned groups were 1.7 and 1.5 days, respectively (Fig. 3).

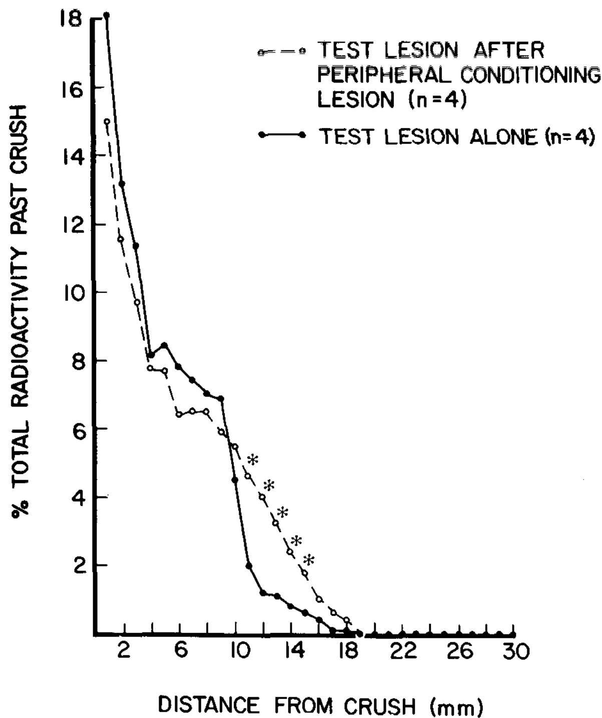

Figure 5. Effect of a conditioning lesion on the population of regenerating axons in the peripheral branch of the DRG at 5 days after test lesions. The percentage of total radioactivity past the crush site contained in each 1-mm segment of peripheral nerve is plotted as a function of distance from the crush (zero distance). *Solid circles* depict mean values from animals that received a test lesion alone, and *open circles* depict mean values from animals that received a conditioning lesion of the sciatic nerve 2 weeks prior to the test lesion. *Asterisks* indicate significant differences ($p < 0.05$) of the group means as assessed by the Student's t test (significant differences more proximal than 10 mm from the crush are not indicated).

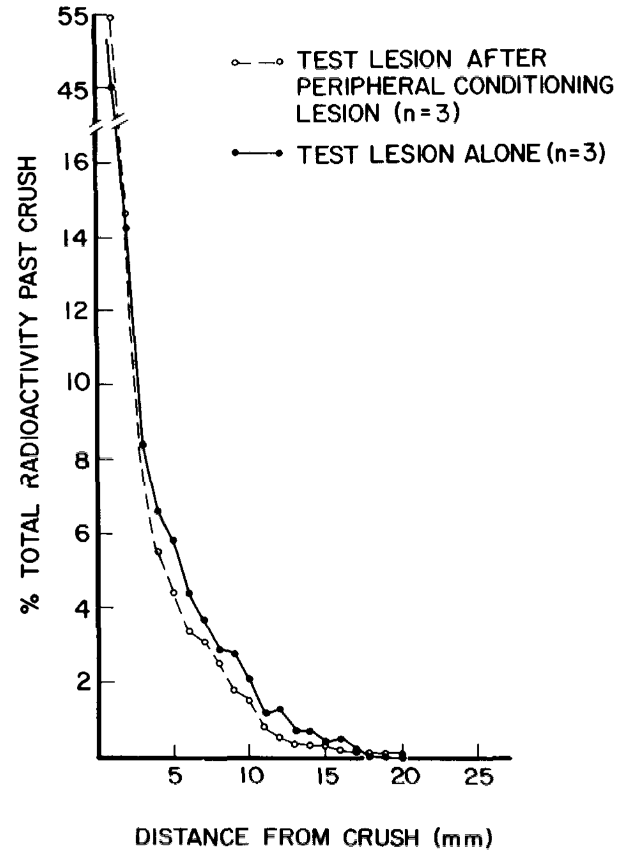

Figure 6. Effect of a peripheral conditioning lesion on the population of regenerating dorsal root axons at 5 days after test lesions. The percentage of the total radioactivity past the crush site of each 1-mm segment is plotted as a function of distance from the crush (zero distance). The *solid circles* depict mean values from the test lesion only group, and the *open circles* depict mean values from animals which had received a conditioning lesion of the sciatic nerve 2 weeks prior to the test lesion of the dorsal root. No significant differences were found at any distance.

Unlike the peripheral branch, the rate of elongation of leading dorsal root axons was not affected by a peripherally placed conditioning lesion made 2 weeks prior to the test lesion. The mean axonal outgrowth distances of the fastest growing axons in the conditioning lesion and test lesion only groups were not significantly different at any time point examined (Fig. 4). The outgrowth of the fastest growing dorsal root axons was linear in both groups, as indicated by the significant correlation coefficients of the linear regression functions of outgrowth distance on time ($r = 0.96, p < 0.05$, both groups, Fig. 4).

The rate of elongation of the fastest growing dorsal root axons in the group of animals that had received a conditioning lesion of the sciatic nerve was 2.6 mm/day; in control animals, this rate was 2.5 mm/day (Fig. 4). This difference was not significant. The initial delay prior to elongation also appeared to be unchanged by a prior conditioning lesion of the sciatic nerve. Initial delay times of 0.4 day for the single lesion and 0.3 day for the conditioning lesion group were estimated by extrapolation of the regression functions to zero outgrowth distance (Fig. 4).

Effect of a conditioning lesion on regeneration of more slowly growing axons in the two branches of DRG cells. After a test crush, not all axons of a nerve regenerate at the rate of the leading axons. In motor axons, it has been reported that a conditioning lesion could affect a populaton of more slowly growing axons without influencing the fastest growing axons (McQuarrie, 1978, 1981). We examined the possibility that a population of regenerating sensory axons trailing behind the fastest growing axons was accelerated by a conditioning lesion. By calculating the total radioactivity past the crush site in a particular nerve and plotting the percentage of this total contained in each 1-mm segment of nerve distal to the crush at 5 days, a profile of labeling in the entire population of regenerating axons that had traversed the crush site at that time was obtained. Data from all animals in a group were averaged.

Figure 5 depicts the results obtained from the group that had received a single test crush of the peripheral branch compared to the group that had sustained a conditioning lesion of the sciatic nerve prior to a test crush. At 5 days after a test lesion, 23% of the total postcrush radioactivity was found between 10 and 20 mm past the crush site in the conditioned group compared to 11% in the test lesion only group (Fig. 5). At several postcrush distances in this range, the percentage of total radioactivity was significantly greater in the conditioning lesion group than in the control group (*asterisks*, Fig. 5). At 7 days after a test crush, similar results were obtained, but the radioactivity extended further distally in both groups (data not shown). The significantly greater amounts of postcrush radioactivity at these distances indicated that more axons had grown further from the crush site in the conditioned group compared to the control group. Therefore, the conditioning lesion had the effect of accelerating outgrowth in a population of regenerating axons that were growing more slowly than the leading axons as well as accelerating outgrowth in leading axons. Since this method does not provide a way of estimating initial delay for the more slowly growing axons, it was not possible to attribute the result to an acceleration of elongation rate. While this is the most likely interpretation, the possibility that delay times were shortened cannot be ruled out.

Unlike the axons of the peripheral branch, the population of dorsal root axons that was growing more slowly than the leading axons appeared to be unaffected by a previous conditioning lesion of the sciatic nerve. As shown in Figure 6, the patterns for the control and the conditioning lesion groups were very similar. In comparing the two groups, no significant differences in relative radioactivity levels were found at any postcrush distance at 5 days (Fig. 6). This result was also obtained at 7 days after crush (data not shown). We concluded that for the central branch DRG axons, a conditioning lesion of the sciatic nerve had no detectable effect on regeneration of the population of more slowly growing axons or, as discussed earlier, on the rate of regeneration of the fastest growing axons.

Discussion

Effect of a conditioning stimulus on regeneration of sensory axons. The effect of a prior lesion on axonal regeneration has been described in several types of neurons in mammals as well as lower vertebrates (reviewed in Grafstein and McQuarrie, 1978; Forman et al., 1981; McQuarrie, 1984). The response of different types of neurons to a conditioning stimulus varies. The most marked response occurs in optic axons of goldfish, where the regeneration rate doubles and the initial delay of regrowth is reduced by half in conditioned axons compared to normal (McQuarrie and Grafstein, 1981). In mammalian motoneurons, a conditioning lesion does not affect the rate of elongation of the fastest growing axons but does accelerate a population of more slowly growing axons (McQuarrie, 1978, 1981).

In rat peripheral sensory axons, sensitive radioisotopic methods had not been previously used to assess the effect of a conditioning lesion on axonal regeneration. However, using the pinch test technique to locate the most rapidly growing sensory axons, McQuarrie et al. (1977) reported a 23% increase in axonal elongation rate with no change in initial delay when a conditioning lesion had been made 2 weeks earlier. In a later study using this same method, the positive response of peripheral sensory axons to a conditioning lesion was attributed to a shorter initial delay with no effect on the rate of axonal elongation (Forman et al., 1980). Our data confirm the results obtained by McQuarrie et al. (1977), since we found a 25% increase in the elongation rate of leading peripheral sensory axons due to a conditioning lesion. Our study provides the additional information that the effect of a conditioning lesion is not limited to just the leading axons, since a population of more slowly growing axons of the peripheral branch was also accelerated by a conditioning lesion.

The effect of a conditioning lesion in the DRG system on regeneration of dorsal root axons had not been previously examined. We found that a lesion of the sciatic nerve 2 weeks prior to a test crush of the dorsal root did not result in a change in either the rate of elongation of leading central branch axons or the initial delay. A population of more slowly growing dorsal root axons was also unaffected by a prior lesion of peripheral branch axons. These results raise two questions: How does a prior peripheral axotomy result in increased rates of elongation of peripheral DRG axons? Why does the central branch axon fail to show a similar response?

Changes originating in the cell body are likely to underlie the conditioning lesion effect. There are two conceivable loci for a conditioning lesion effect. One is the nerve itself, as originally proposed by Gutmann et al. (1942). They suggested that events such as increased Schwann

cell proliferation brought about by multiple nerve injury may underlie the conditioning effect. However, the large distance between the site of the conditioning and testing axotomies used in our study makes this explanation somewhat unlikely. Additionally, it is known that a prior axotomy is exhibited even when axons are forced to regrow into connective tissue rather than through their original nerve sheaths by excising a length of peripheral nerve (McQuarrie and Grafstein, 1973; McQuarrie, 1979). The effect is also expressed when conditioned neurons are placed in culture (Agranoff et al., 1976; Landreth and Agranoff, 1976, 1979). These and other observations argue that the conditioning effect is not directly mediated through the local environment surrounding the axons, and they lend considerable support to the second possibility that metabolic changes arising in the nerve cell body are responsible (Grafstein and McQuarrie, 1978; Forman et al., 1981).

Axotomy of the peripheral DRG axon provides a signal adequate to elicit a cell body response, while dorsal root injury does not. For example, damage to the peripheral branch of DRG cells has long been known to induce chromatolysis and changes in protein synthesis in spinal ganglion cells, while dorsal root section does not produce such cell body changes (reviewed in Cragg, 1970; Lieberman, 1971; Perry and Wilson, 1981; Hall, 1982). Although the nature of the signal created by peripheral axotomy that induces such cell body changes is unknown, it is most probably conveyed to the cell by retrograde transport (Bisby, 1982). Since the effectiveness of a prior axotomy appears to depend on the initiation of a cell body response, it is likely that a conditioning lesion made on the dorsal root would not be an effective way to affect the regeneration rate of either axonal branch. However, in the experiments we report here, the conditioning axotomy was always made on the peripheral branch, and the lack of an effect on subsequent regeneration in the dorsal root cannot be simply attributed to an inadequate signal to the DRG cells. Instead, changes occurring in the DRG neurons appear to be selectively expressed. In order for changes in cell body protein synthesis to affect one or both of the DRG axons, axonal transport is required, since this process provides all of the materials axons require for maintenance and growth (reviewed by Lasek and Hoffman, 1976; Grafstein and Forman, 1980; Lasek, 1981).

Possible role of fast axonal transport in regeneration of DRG axons after a conditioning lesion. During regeneration, new axolemmal constituents are provided by fast axonal transport (Griffin et al., 1976, 1981; Tessler et al., 1980). Some aspects of the regeneration process, particularly the time course of the initial phase of sprout formation, may be quite dependent on fast axonal transport. One of the reported conditioning lesion effects in other systems is a shortening of the initial delay times for regeneration (reviewed by Forman et al., 1981). In the goldfish optic nerve, a reduction in the initial delay as a result of a conditioning lesion is associated with changes in the amounts of rapidly transported protein (McQuarrie and Grafstein, 1982).

In our study of the sensory system, we found no significant changes in the initial delay in either branch of the DRG cell as a result of a conditioning lesion. Bisby (1978, 1981) has noted the lack of significant changes in the amount of fast transported protein in both branches of DRG cells in response to a single peripheral axotomy. In fact, only a transient decrease in the amount of protein exported at fast rates, which returned to normal by 14 days after axotomy, was found to occur coordinately in the two branches of DRG cells (Bisby, 1981). While quantitative routing of fast axonally transported proteins is known to occur normally in the DRG system (Ochs et al., 1978), Bisby (1981) has suggested that this process is not sensitive to changes induced by lesioning one branch. Therefore, it is unlikely that changes in fast transport underlie the selective conditioning lesion effect in our study.

Changes in slow axonal transport are likely to mediate altered regeneration of DRG axons after a conditioning lesion. A likely candidate underlying the conditioning lesion effect is a change in slow axonal transport. Since slow axonal transport represents the movement of major cytoskeletal elements as well as the axoplasmic matrix (Tytell et al., 1981; Brady and Lasek, 1982), its role in providing materials necessary for regeneration is clearly established (Lasek et al., 1981; McQuarrie, 1983). The rate of movement of SCb correlates with that of axonal elongation (Lasek et al., 1981). For example, in the DRG cell, both the rate of regeneration and the rate of SCb are slower in the central branch than in the peripheral branch but are similar within the same branch (Mori et al., 1979; Komiya, 1981; Wujek and Lasek, 1983). The correlation also holds in rat ventral motoneurons where the rate of regeneration (Griffin et al., 1976; Forman and Berenberg, 1978; Bisby, 1979; Black and Lasek, 1979, Pestronk et al., 1980) and the rate of SCb (Hoffman and Lasek, 1975; Lasek and Hoffman, 1976) are similar.

Lasek et al. (1981) have proposed that slow axonal transport of the cytoskeleton, particularly SCb, is a primary rate-limiting process in axonal elongation. Wujek and Lasek (1983) have further suggested that the difference in the rate of regeneration of the two axonal branches of the DRG cell is fundamentally determined by the difference in the rate of SCb in the two branches because SCb may contain the primary motile component essential for translocating the cytoskeleton of axons and their growth cone. Support for these hypotheses is found in the fact that factors which change the rate of axonal regeneration coordinately change the rate of SCb. For example, during development both have been shown to decrease (Black and Lasek, 1979; Komiya, 1980, 1981; Pestronk et al., 1980) and changes in temperature concomitantly change the rate of SCb and regeneration (Cancalon, 1983a, b).

If the hypothesis that axonal elongation is in large part dependent on the rate of movement of SCb is correct, then an increase in regeneration rate that occurs in response to a conditioning lesion should be accompanied by an increase in the rate of SCb. In fact, this has been recently documented in the goldfish optic system (McQuarrie and Grafstein, 1982). Presumably, some change in the composition of SCb, qualitative and/or quantitative, would underlie the rate change in SCb. We have begun to examine the changes in slow transport in the two branches of the DRG cell that occur in response to axotomy in the DRG system (Oblinger and Lasek,

1983), but further work is required to clarify the underlying changes in slow axonal transport that may be the basis for the conditioning effect.

Possible role of environmental factors in central axon regeneration. In considering why the central branch axon of the DRG cell does not exhibit an accelerated regeneration rate after a peripheral priming lesion, one possibility involves factors extrinsic to the neuron. For example, is the environment of the dorsal root the predominant factor limiting the rate of axonal elongation of central branch axons? If so, it is conceivable that even if changes in slow (or fast) transport occurred coordinately in both branches of the DRG cell axon after a peripheral conditioning lesion, an effect on regeneration rate would not be manifest in the dorsal root. While our data do not negate this possibility, a recent study provided information relevant to this issue. Wujek and Lasek (1983) found that the rate of regeneration of axons of ventral motoneurons through the ventral root was identical to the rate at which they elongate in the sciatic nerve. By histological criteria, the ventral and dorsal roots are very similar (Haller and Low, 1971; Haller et al., 1972). It is difficult to attribute differences in the regeneration rate of motor axons in the ventral root (4.6 mm/day) from the regeneration rate of DRG axons in the dorsal root (2.5 mm/day) only to presumptive differences in the environment of ventral and dorsal roots. However, experiments that more directly address this issue for the DRG cell involves cross-grafting paradigms and have not yet been reported.

An alternative criteria for negating the role of the root environment as the predominant factor limiting the rate of central axon regeneration would be the demonstration of a positive response of the central DRG axon by a stimulus delivered to the cell body by the central branch. A prior lesion of the dorsal root axon does not appear to alter the regeneration rate of the central axons (M. M. Oblinger and R. J. Lasek, unpublished data). However, a central axotomy also does not produce a detectable cell body response or changes in protein synthesis in the DRG cell and may not be an appropriate stimulus for studying this question. Future studies may provide information relevant to this issue.

Does the DRG cell separately organize the cytoskeleton of each axonal branch? If we postulate that differences in the environments of central and peripheral branches of the DRG cell are not sufficient to account for differences in their regeneration rates or the selective effect of a peripheral conditioning lesion, the major alternative is that the DRG cell can separately regulate the properties of each axon. By what mechanism could this be done? A useful construct in considering this issue is the proposal that the two "organizing regions" that appear to give rise to the original processes of DRG cells in their bipolar history are preserved through ontogeny and remain separate (Lasek, 1981). DRG cells begin life in the embryo as bipolar cells with two entirely separate neurites that, in subsequent development, associate to form a single stem axon that bifurcates (reviewed in Tennyson, 1965; Pannese, 1974). Electron microscopic studies in the adult system indicate an independent course of microtubules and neurofilaments passing from the stem axon to each branch of the bifurcation, suggesting a continuity of the cytoskeleton of each branch from the cell body (Ha, 1970).

The hypothesis that the DRG cell body separately organizes the cytoskeletal networks of its peripheral and central branches requires that transported proteins are segregated into two compartments at some point along the pathway between synthesis and entry into the axons. This raises the possibility that assembly and transport of cytoskeletal proteins into the peripheral and central cytoskeletal networks could be separately regulated. Changes in cell body protein synthesis, at least for the cytoskeletal proteins, could be unequally expressed in the two cytoskeletal networks. Clearly, if this were the case, some of the functional properties that are fundamentally related to cytoskeletal transport, such as regeneration rate, could also be unequally affected. The results of our experiments are consistent with this and suggest that changes in protein synthesis during the chromatolytic response to peripheral axotomy are expressed through the cytoskeletal network of the peripheral axon but do not effect the cytoskeletal network of the central axon.

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