# Degenerating Nerve Fiber Products Do Not Alter Physiological Properties of Adjacent Innervated Skeletal Muscle Fibers

Abstract. *Partial denervation of rat extensor digitorum longus and soleus muscles resulted in the appearance of denervated and innervated surface muscle fibers lying adjacent to one another. The denervated muscle fibers showed the typical signs of denervation while the innervated muscle fibers were similar to those of control muscles. We conclude that denervated fibers do not induce substantial physiological changes in adjacent innervated muscle fibers.*

The maintenance of the morphological and physiological properties of the skeletal muscle fiber is dependent on the integrity of its innervation. The precise mechanisms underlying this neural influence remain obscure but, in addition to neuromuscular transmission (*1*), apparently include the release of trophic substances from the nerve terminal (*2*).

This interpretation has been questioned by Jones and colleagues (*3*). These authors have suggested that inflammation associated with nerve degeneration rather than loss of trophic influences may cause denervation changes in muscle since (i) implantation of a piece of either degenerating nerve or silk thread onto rat extensor digitorum longus (EDL) or soleus muscles causes local inflammation which is accompanied by a decrease in resting membrane potential (RMP) and an increase in acetylcholine (ACh) sensitivity of the underlying normally innervated muscle fibers; (ii) denervation of rat soleus muscle leads to the appearance of inflammatory cells along the entire length of the muscle fibers; and (iii) treatment with inhibitors of cell division such as vincristine or fluorouracil prevents both the proliferation of inflammatory cells as well as the increase in ACh sensitivity which normally follow nerve section.

Cangiano and Lutzemberger (*4*) in extending this concept reported that partial denervation of the EDL muscle in rats results in the appearance of tetrodotoxin (TTX)-resistant action potentials, a well-known effect of denervation, not only in the denervated muscle fibers but also in the adjacent innervated muscle fibers as well. They concluded (i) that nerve degeneration is responsible for some changes in denervated muscle fibers; (ii) that products of nerve degeneration diffuse to and cause denervation changes in adjacent innervated muscle fibers; and (iii) that this process along with the cessation of nerve impulse transmission can account for all of the changes in skeletal muscle properties caused by denervation. Their interpretations thus cast serious doubt on the idea that normal muscle properties are also maintained by neurotrophic substances.

Because of the importance of the neurotrophic hypothesis, we have reexamined Cangiano and Lutzemberger's proposal in more detail by partially denervating both EDL and soleus muscles of female Wistar rats (200 to 250 g). Partial denervation was accomplished by two methods. In the first method, either spinal nerve L4 or L5 (*5*) was completely sectioned 2.0 to 2.5 mm from the intravertebral foramen through a dorsolateral incision at the lumbar region in rats anesthetized with chloral hydrate (400 mg/kg, given intraperitoneally). In the second method, the sciatic nerve branch supplying the EDL was exposed 1.0 to 1.5 cm from the EDL muscle while the animals were under ether anesthesia; approximately half of this branch was then crushed. Both surgical treatments were performed on the right side, and the contralateral muscles served as unoperated control muscles. The RMP, extrajunctional sensitivity to ACh, and TTX-resistance of directly elicited action potentials of surface fibers were assessed 48 to 216 hours after the operation, most experiments being done 54 to 84 hours after surgery.

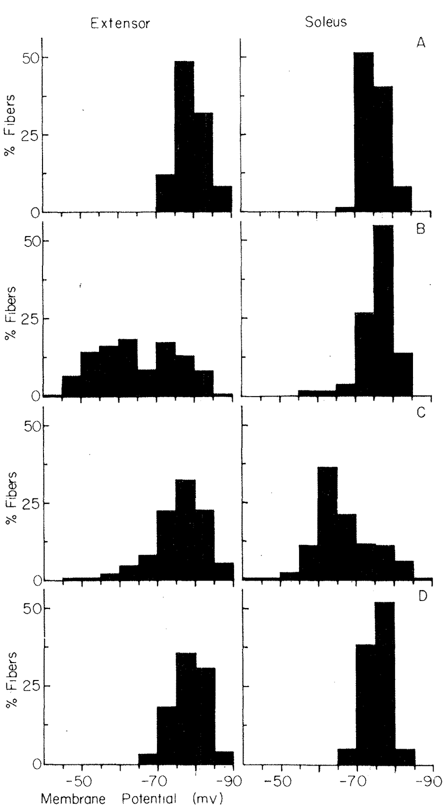


Fig. 1. Frequency histograms of the resting membrane potential of surface fibers of EDL and soleus muscles. (A) Contralateral control muscles. (B) Partially denervated muscles 48 to 216 hours after L4 section. (C) Partially denervated muscles 72 to 120 hours after L5 nerve section. (D) Muscle fibers present in partially denervated muscles displaying MEPP's where either L4 section, L5 section, or partial sciatic nerve crush was performed.

Examination of the surface fibers of the partially denervated EDL or soleus muscles revealed typically denervated fibers lying adjacent to normal innervated ones (Tables 1 and 2; Figs. 1 and 2). Transection of spinal nerve L4 or partial crushing of the nerve supplying the EDL caused an average decrease in RMP of 67 and 49 percent of EDL surface fibers, respectively (Table 1). Transection of L5 had less effect on the EDL but did cause, on the average, a decreased RMP in 67 percent of soleus surface fibers (Table 1). Figure 1 shows that fibers with normal RMP and fibers with low RMP were both present in partially denervated muscles. Furthermore, the two kinds of fibers, those with normal and low RMP, were found adjacent to one another. The surface fibers that were partially depolarized appeared to be denervated because spontaneous miniature end-plate potentials (MEPP's) were not detected and nerve stimulation failed to elicit either end-plate potentials or action potentials at the junctional muscle membrane. The other group of muscle fibers appeared to be normally innervated because their RMP's were similar to those of control unoperated muscles, they had MEPP's of normal amplitude and frequency (Table 2), and they all responded with an action potential and twitch to nerve stimulation.

Examination of extrajunctional ACh sensitivity (*7*) of partially denervated EDL and soleus muscles gave a similar result. Marked extrajunctional ACh sensitivity was found along the entire surface in each fiber examined of the EDL and soleus muscles which was partially depolarized and without MEPP's, whereas fibers with normal RMP's and MEPP's never showed increased extrajunctional ACh sensitivity (Table 2).

The innervated and denervated muscle fibers of the partially denervated muscles were also compared with respect to the action of TTX ($1 \times 10^{-6}M$) in blocking the muscle action potential (*8, 9*). Again, two distinct groups of muscle fibers were found. In the denervated muscle fibers (those with low RMP's and without MEPP's), TTX only partially blocked the muscle action potential and overshooting action potentials were commonly observed (Fig. 2). However, in-

Table 1. Effect of partial denervation on resting membrane potential.

| Treatment | RMP (mv) of cells with: MEPP's* | RMP (mv) of cells with: Low RMP† | Fibers with low RMP (%): Mean | Fibers with low RMP (%): Range |
|---|---|---|---|---|
| EDL | | | | |
| Control | − 78.5 ± 0.3 | | | |
| Partial nerve crush | − 77.9 ± 0.5 | − 53.7 ± 1.0 | 49 | 30 to 60 |
| L4 section | − 78.0 ± 0.6 | − 58.1 ± 0.4 | 67 | 45 to 95 |
| L5 section | − 77.1 ± 0.6 | − 63.5 ± 0.8 | 16 | 5 to 25 |
| Soleus | | | | |
| Control | − 73.8 ± 0.4 | | | |
| L4 section | − 74.8 ± 0.5 | − 63.1 ± 1.7 | 5 | 0 to 8 |
| L5 section | − 74.1 ± 0.6 | − 61.6 ± 0.3 | 67 | 45 to 95 |

*Data (mean ± S.E.M.) represent 23 to 190 muscle fibers from 4 to 13 muscles. †Average RMP (mv) of those cells whose RMP was more than two standard deviations below the mean RMP of contralateral control muscles.

Table 2. Effect of partial denervation on MEPP's, extrajunctional ACh sensitivity, and directly elicited action potentials.

| Treatment | MEPP*: Amplitude (mv) | MEPP*: Frequency ($sec^{-1}$) | ACh sensitivity (mv/nanocoulomb): Innervated‡ | ACh sensitivity (mv/nanocoulomb): Denervated§ | Fibers with overshooting action potentials in presence of $1 \times 10^{-6}M$ TTX†: Innervated (%) | Fibers with overshooting action potentials in presence of $1 \times 10^{-6}M$ TTX†: Denervated (%) |
|---|---|---|---|---|---|---|
| EDL after L4 section | 0.53 ± 0.06 | 2.8 ± 0.1 | < 0.001 | 92.5 ± 10.7 | 0 | 95 to 100 |
| Soleus after L5 section | 0.49 ± 0.08 | 1.2 ± 0.2 | < 0.01 | 115.0 ± 22.2 | 0 | 95 to 100 |

*The values shown for MEPP amplitude and frequency were not significantly different from those of control muscles. The data are means ± S.E.M. of at least ten surface fibers from at least three muscles. †Approximately 20 percent of the innervated muscle fibers displayed an action potential which did not reach zero potential (see Fig. 2). ‡Innervated fibers refer to fibers which displayed MEPP's and normal RMP. §Denervated fibers refer to those fibers which were lacking MEPP's and were partially depolarized.

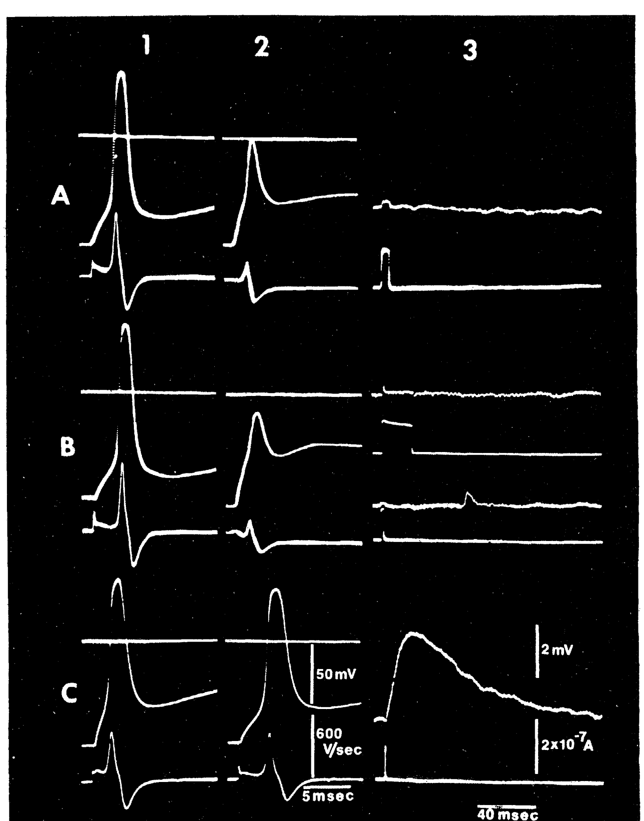


Fig. 2. Typical records of directly elicited action potentials and extrajunctional ACh sensitivity of EDL muscles. (A) Contralateral control muscle fibers. (B) Innervated muscle fibers of partially denervated muscles. (C) Denervated muscle fibers of partially denervated muscle. 1. Action potentials elicited in normal Ringer solution. 2. Action potentials elicited in presence of $1 \times 10^{-6}M$ TTX. In each panel of 1 and 2, the upper trace indicates zero membrane potential and the middle and bottom traces show the action potential and its rate of rise ($dv/dt$), respectively. 3. ACh sensitivity of the extrajunctional muscle membrane. For each pair of traces, the upper one shows the membrane response to the microiontophoretically applied ACh. The bottom one shows the current applied to the ACh pipette. For the lower pair of traces in B3 the recording electrode was close to the end plate region (note MEPP) but the iontophoretic pipette was placed 200 to 300 μm from the end plate region so that junctional ACh sensitivity would not be observed. The ACh potential shown in C3 was elicited by a current pulse that was 0.2 msec in duration.

nervated muscle fibers (those with normal RMP's and MEPP's) did not show overshooting action potentials. In fact, in most of the innervated fibers the phasic potential elicited by the current pulse junctionally as well as extrajunctionally could be attributed to delayed rectification by the muscle membrane of the applied square current pulse. In many muscle fibers though (20 percent), TTX was unable to block completely the action potential of the innervated surface fibers of partially denervated EDL and soleus muscles. However, this behavior was observed similarly in the normally innervated EDL. Therefore, the innervated muscle fibers did not acquire resistance to the blocking action of TTX in the manner observed in denervated muscles (Fig. 2) (*9*). In addition, it may be expected that the presence of TTX-resistant action potentials at the muscle end plate is not necessarily a sign of denervation since Thesleff *et al.* (*10*) have shown that TTX-resistant action potentials commonly occur in innervated end plates of the rat diaphragm.

We have found that partially denervated EDL and soleus muscles display two distinct classes of surface muscle fibers: one class which represents denervated fibers with denervation changes similar to those which have been established for completely chronically denervated muscle, and a separate class which represents remaining normally innervated muscle fibers whose membrane properties do not differ substantially from control unoperated muscle fibers. These innervated and denervated fibers were commonly found adjacent to one another. Furthermore, even in those preparations that were predominantly denervated, any cell which displayed MEPP's always had a RMP, extrajunctional ACh sensitivity, and TTX-sensitivity comparable to control muscle fibers. These results show that innervated and denervated fibers can exist next to each other without the innervated fibers developing any obvious signs of denervation. This finding, therefore, does not support the suggestion (*4*) that nerve degeneration products resulting from partial denervation of a muscle induce denervation-like changes in the remaining innervated fibers.

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5. Spinal nerves L4 and L5 were designated as described by E. C. Greene [*The Anatomy of the Rat* (American Physiological Society, Philadelphia, 1935; Hafner, New York, reprint, 1968)]. The more rostral of the two spinal nerves comprising the sciatic nerve is therefore referred to as L4, whereas Cangiano and Lutzemberger (*4*) refer to this nerve as L5.
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7. Extrajunctional ACh sensitivity was determined by microiontophoretic application of ACh, with high-resistance glass micropipettes (150 to 300 megohms) filled with 2.5*M* acetylcholine chloride. Current pulses were $2 \times 10^{-8}$ to $6 \times 10^{-7}$ amp and 0.05 to 20.0 msec in duration. For the innervated muscle fibers, current pulses of 5 to 20 msec in duration were routinely used in order to assess adequately the presence of measurable extrajunctional ACh sensitivity.
8. Directly elicited action potentials were obtained by inserting two microelectrodes into the same surface fiber 50 to 100 $\mu$m apart, one to pass current and the other to record the voltage response. The RMP was set at −90 mv to obtain more homogeneous responses by applying a hyperpolarizing current through the current electrode [T. Narahashi, *J. Cell. Comp. Physiol.* **64**, 73 (1964); E. X. Albuquerque and S. Thesleff, *Acta Physiol. Scand.* **73**, 471 (1968)]. In many cells, the depolarizing current pulse was also delivered with the RMP set at −120 mv to ensure that the pattern of TTX-resistance which we observed was not dependent on the membrane holding potential. The effect of TTX ($1 \times 10^{-6}M$) on directly elicited action potentials was then compared in the two classes of surface muscle fibers to determine whether the action potentials of innervated muscle fibers of partially denervated muscles develop resistance to the blocking action of TTX in a similar way to that observed in denervated muscle fibers.
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## Phototoxic Keratoconjunctivitis from Coal-Tar Pitch Volatiles


Abstract. *Roofers working with coal-tar pitch develop burning eyes and conjunctivitis which they subjectively associate with sun exposure. A coal-tar pitch distillate instilled in the conjunctivae of rabbits produced minimal or mild irritation in the absence of ultraviolet radiation, but irradiation with long-ultraviolet produced marked photophobia and severe keratoconjunctivitis.*


Photosensitization reactions induced by exogenous agents have not been clearly shown to cause human eye disease. In this report we describe observations on humans employed in the roofing trade and on New Zealand white rabbits which demonstrate that ultraviolet (UV) radiation of wavelengths present in sunlight, and volatiles from coal-tar pitch, can act in concert to produce injury to the cornea and conjunctiva (keratoconjunctivitis). This injury appears analogous to phototoxic injury to the skin from coal-tar pitch and sunlight (*1*, *2*).

Two groups of U.S. roofers occupationally exposed to coal-tar pitch were examined. Six of 34 roofers in one group and 11 of 17 roofers in the other had clinical signs of keratoconjunctivitis. There were statistically significant ($P < .05$, Fisher's exact test) correlations between the presence of conjunctivitis on examination and occupational exposures to airborne polycyclic particulate organic matter of $\geq 0.20$ and $\geq 0.18$ mg/m$^3$, respectively, for the two groups (*3*). In each case the personal exposures represented a time-weighted average value for the entire work period. Affected roofers complained of severe burning in the eyes on exposure to the sun. In addition, the majority of roofers in each group gave a history of severe episodes of probable keratoconjunctivitis in the past, which they related to exposure to pitch vapors or dusts. Other authors have described both acute and chronic ocular changes as a result of human exposure to coal-tar pitch or its components (*1*, *4*, *5*). Susorov (*5*) noted that eye irritation in workers unloading pitch was more pronounced when the work was carried out in clear sunlight.

Table 1. Subjective assessment by 11 white journeyman roofers of the relative influence of environmental variables and different roofing materials on the development of skin and eye symptoms. Symptoms were scored as very bad, 5; bad, 3; occasional, 1; and none, 0.

| Variable | Mean score: Eye symptoms | Mean score: Skin symptoms |
|---|---|---|
| Sunny day | 3.9 | 2.5 |
| Summer | 3.7 | 2.7 |
| Windy day | 2.9 | 2.7 |
| Very humid | 2.8 | 2.3 |
| Still day | 2.0 | 1.8 |
| Not humid | 1.4 | 1.3 |
| Winter | 1.0 | 1.0 |
| Cloudy day | 0.9 | 1.0 |
| Pitch (about 260°C) | 3.6 | 2.5 |
| Pitch (about 200°C) | 2.2 | 1.6 |
| Asphalt | 0.1 | 0.1 |
| Gravel | 0.2 | 0.1 |

Fifteen journeyman roofers rated from personal experience the relative influence of certain environmental variables in producing eye and skin symptoms. Selected ratings obtained from 11 white males are shown in Table 1. Although only the skin manifestations are generally considered phototoxic, subjective associations with sunny days or summer were stronger for eye symptoms than for skin symptoms. Four black male roofers gave rankings for eye symptoms similar to those of the white roofers, but only one black reported any skin symptoms. Both eye and skin symptoms were clearly associated with the use of coal-tar pitch rather than other roofing materials and were more pronounced when pitch was used at a higher temperature, where more would be volatilized.

As these observations suggested a role for photosensitization in producing eye irritation, the results of controlled exposures of the eye to UV and pitch alone and in combination were examined in New Zealand white rabbits. The animals weighed between 3 and 4 kg and were maintained in UV-free quarters. Thirty minutes before treatment acepromazine (2 mg/kg) was given subcutaneously and eyelashes were trimmed to a length of 3 mm. One eye of each rabbit was exposed to radiation from a bank of BLB40 fluorescent black lights (predominantly 330 to 380 nm), passed through a window-glass filter to remove radiation below 320 nm. Total radiant exposure was controlled by using a photodosimeter. During irradiation the other eye was covered with an opaque shield and reflective surfaces in the room were covered with black cloth. Roofing coal-tar pitch volatiles were prepared by collecting vapors from a large sample maintained at 200°C, using Romovacek's method (*6*). Gross and biomicroscopic observations of the eyes were made 5, 24, 48 and 72 hours after treatment (longer if abnormalities persisted) by an observer who did not know how each eye had been treated.

Preliminary studies showed that rabbits irradiated shortly after conjunctival instillation of 10 $\mu$l of coal-tar pitch distillate to both eyes would tightly close only the irradiated eye within about 3 minutes ($4 \times 10^2$ joule/m$^2$ UV) and keep that eye closed until irradiation was ceased. As a result it was necessary to use an eye speculum to keep the lids open during irradiation.

Each final evaluation group consisted of six rabbit eyes. In all instances both conjunctival sacs of a rabbit were instilled with 10 $\mu$l of either pitch volatiles or distilled water and one eye was subsequently irradiated with UV at $2.0 \times 10^3$ joule/m$^2$. No changes were observed in