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Innervation of Muscle Receptors in the Cross-Reinnervated Soleus Muscle of the Cat

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ABSTRACT It has recently been reported (Gregory et al., J. Physiol., 331:367–383, 1982) that cutting a muscle nerve and letting it grow back into the muscle or cross-uniting the muscle with a foreign nerve results in major disruption of the normal response patterns of muscle spindles and tendon organs. Here we report observations on the structure of muscle receptors in cross-reinnervated and self-reinnervated soleus muscles in an attempt to detect abnormalities that might account for their disturbed function. Eight soleus muscles were reinnervated with the extensor digitorum longus nerve for periods up to 449 days and two were self-reinnervated. Following the physiological investigation, the muscle was fixed and stained according to the method of Barker and Ip (J. Physiol., 69:73P–74P, 1963). Spindles and tendon organs were teased from the muscle and photographed. In one cross-reinnervated muscle an attempt was made to isolate all receptors. About two-thirds of the normal number of spindles and tendon organs were found. Three categories of receptor were identified: normal, abnormal, and those having no visible nerve endings. There appeared to be little difference in degree of abnormality of receptors in self- and cross-reinnervated muscles. Of the 180 spindles, 3% were normal, 43% had no visible endings, and 54% had abnormal endings. Of 80 tendon organs, 38% were normally innervated, 33% were without visible innervation, and 29% had abnormal endings. We conclude that following long-term cross-reinnervation and self-reinnervation of soleus there is extensive disruption of the normal innervation pattern of both spindles and tendon organs which could account for their functional abnormalities.

When a mammalian slow-twitch muscle is denervated and the nerve of a fast-twitch muscle made to grow into it, the mechanical properties of the muscle are transformed to those resembling more closely the fast-twitch muscle (Buller et al., 1960). It remains uncertain whether muscle afferents are involved in the transformation process. In recent years evidence has accumulated indicating that nerve injury, be it nerve crush, freezing, or section, results in a permanent disruption of the innervation of at least some muscle receptors (Quick and Rogers, 1983; Barker and Milburn, 1984; Banks et al., 1985; Scott, 1985). The possibility therefore arises that changes in muscle mechanical properties accompanying nerve cross-union are simply the result of disruption of receptors and the abnormal patterns of activity to which they give rise.

As a first step in the evaluation of the role of muscle receptors in this process, we studied the responses of muscle afferents from cross-reinnervated and self-reinnervated muscles (Gregory et al., 1982). We encountered many response patterns considered as abnormal on our criteria, but at the time we had no information about structural abnormalities that might account for them. Here we report the results of a systematic study of the structure of muscle receptors in cross-reinnervated and self-reinnervated muscle, making particular reference to the pattern of innervation.

MATERIALS AND METHODS

Operations were performed on 12- to 14-week-old male and female cats under Nembutal anesthesia. Fine silk thread (10/0) was tied around the soleus nerve at two points 2–3 mm apart close to where the soleus nerve enters the muscle. The extensor digitorum longus (EDL) nerve was similarly tied at the point of entry to the peroneal muscles. Both nerves were cut between the ties and the cut end of the foreign nerve brought into close apposition to the stump of the original nerve. The nerves were secured end-to-end by the attached ties. Eight cats were cross-reinnervated; in a further two animals a similar operation was performed but the nerves were reunited with their original muscles. We would like to emphasize that the cut ends of the nerves were simply tied together; no special effort was made to maintain fascicular realignment nor did we repair the nerve with epineural sutures (cf., Banks et al., 1985).

Animals were used for the physiological studies between 227 and 449 days postoperatively. At that age they weighed 2.2–3.8 kg. Anesthesia was induced with pentobarbitone sodium (40 mg/kg) and on completion of

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the physiological measurements the animals were killed with an overdose of anesthetic. The soleus muscle on the operated side was excised, and blotted to remove the liquid paraffin oil in which it had been immersed during the physiological experiment. This prevented drying of the tissues and allowed for electrical recording. It was then fixed in chloral hydrate and stained with silver according to the modified impregnation method of Barker and Ip (1963). The stained muscles were stored in glycerine that was changed several times. The muscles were teased under a binocular microscope (approx. 32×), and isolated muscle spindles and tendon organs were mounted in glycerine for microscopic examination.

RESULTS

Observations were made on eight cross-reinnervated, two self-reinnervated, and two control muscles. For one cross-reinnervated muscle the entire muscle was systematically teased into small portions and each piece was searched for muscle receptors. This muscle yielded a total of 21 tendon organs and 39 spindles, which represents about two thirds of the number in a normal muscle (Boyd and Davey, 1968). In the remaining muscles only the region close to the point of nerve entry into the muscle was studied in detail. Altogether, a total of 295 muscle receptors were isolated. Of these, 94 were tendon organs and 201 were spindles. Receptors were classified as normal or abnormal according to the pattern of innervation. Spindles, in which spirals or coils of the afferent fiber around the primary region of the nuclear bag fibers could not be found, were classified as abnormal. These could be further subdivided into those devoid of any afferent ending at all and others containing a few fine, tapering axons running parallel to the intrafusal bundle. On some occasions annulospiral endings were incompletely stained, but whenever there was any trace of a spiral the sensory ending was considered normal. Uneven staining was a problem in this investigation and in several muscles, including controls, a number of receptors were classified as having "no visible ending," presumably because of incomplete staining. As a result, some spindles could have been erroneously placed in this category. However, the striking feature of the reinnervated muscles that distinguished them from the controls was the large number of endings with visible abnormalities.

Muscle Spindles

A total of 201 spindles was examined, 21 from the two control muscles (of which 5 were incompletely stained), 34 from self-reinnervated muscles, and 146 from cross-reinnervated muscles. There was no detectable difference in the degree of abnormality of spindles from self-reinnervated and cross-reinnervated muscles.

Of the spindles in the cross- and self-reinnervated muscles 5 (3%) were normal, 78 (43%) had no visible endings, and 97 (54%) had abnormal endings. The numbers of different spindle types found in the cross-reinnervated, self-reinnervated, and normal muscles are shown in Table 1. Examples of spindles with no apparent sensory endings are shown in Figures 1 and 2. In Figure 1, although several axons were seen approaching the intrafusal bundle, they ended abruptly at a point beyond which no ending of any type could be detected under the microscope used with a 40× objective. The more common pattern was a clearly abnormal afferent innervation. The degree of abnormality varied considerably between receptors. Often there were no visible spiral terminations around the intrafusal fibers but a diffuse network of fine axon branches (Figs. 3, 6) that tapered to terminate as threads (Fig. 8) or a series of varicosities (Figs. 9, 10). On other occasions annulospiral endings could be clearly recognized but with abnormal fine branching endings on adjacent regions of the intrafusal fibers (Fig. 4). In some cases motor terminals nearly normal in appearance could be identified in polar segments of the intrafusal fibers (Fig. 7). A spindle from a control muscle, with a typical annulospiral ending clearly visible, is shown in Figure 5.

TABLE 1. Summary of the types of spindles found in the reinnervated and control muscles

	Spiral (normal)	Abnormal or plexus	No visible ending
Cross-reinnervated	5	61	78
Self-reinnervated	0	34	0
Control	9	5	7

TABLE 2. Summary of the types of tendon organs found in the reinnervated and control muscles

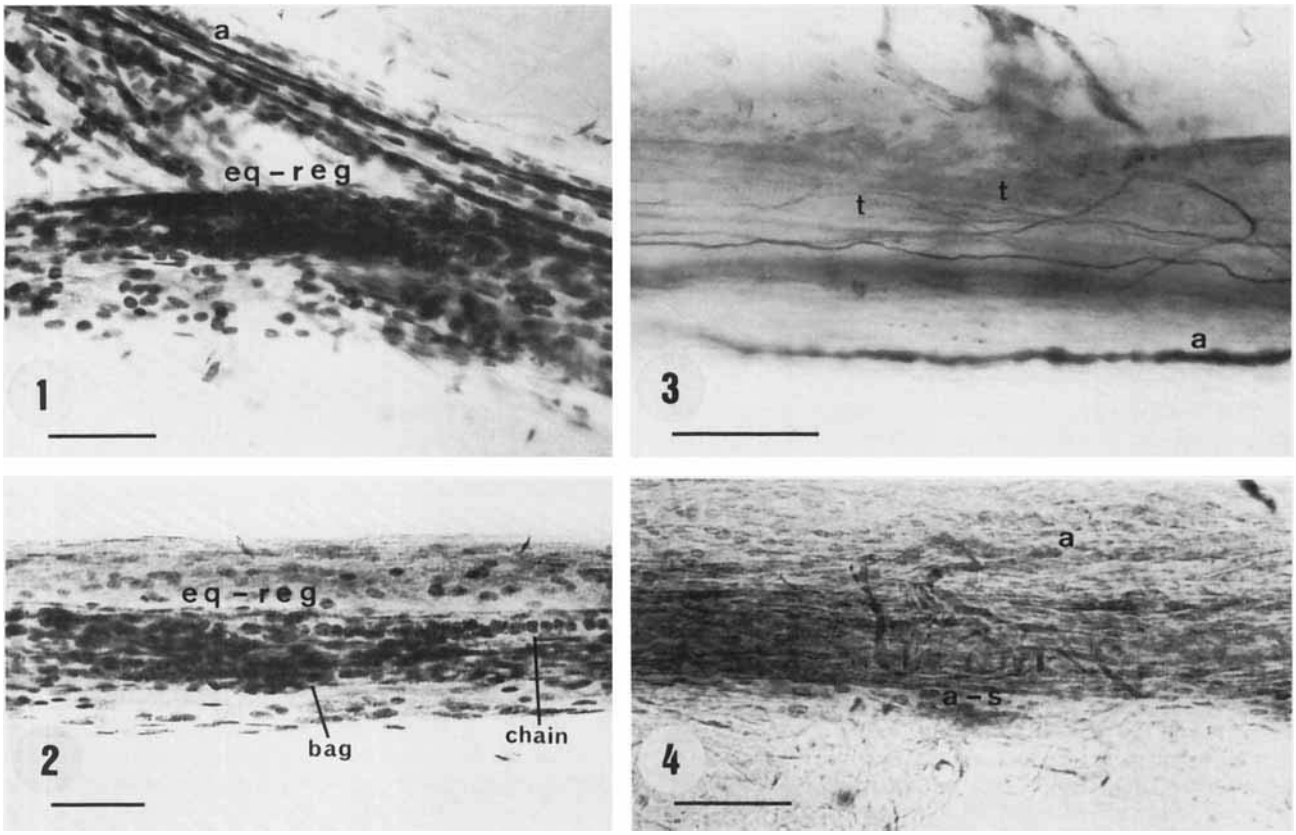
	Normal	Abnormal or plexus	No visible ending
Cross-reinnervated	29	19	25
Self-reinnervated	2	4	1
Control	7	0	7

Tendon Organs

The reinnervated muscles yielded a total of 80 tendon organs. Of these, 31 (38%) appeared to have normal innervation, 26 (33%) had no visible innervation at all, and 23 (29%) had abnormal endings. The numbers of different tendon organ types found in the cross-reinnervated, self-reinnervated, and normal muscles are shown in Table 2. The pattern of disruption resembled that seen in spindles, except that there were many more examples of a normal innervation.

An example of a tendon organ devoid of any visible innervation is shown in Figure 12. Another example, from a cross-reinnervated muscle, with an extensively branching network of terminals is shown in Figure 13 and under higher power in Figure 14. These endings should be compared with the pattern seen in a receptor from a normal muscle (Fig. 11).

In those spindles or tendon organs in which afferent axons were clearly visible at their point of entry into the receptor capsule, the axon diameter was measured at the entry point. Measurements, each a mean of three internodal readings, were made with a micrometer eyepiece and 40× objective. (Magnification was calibrated with a graduated micrometer slide, allowing eyepiece readings to be converted to microns). The distribution of fiber diameters from cross-reinnervated, self-reinnervated, and control muscle afferents are shown in Figure 15. Axonal fiber diameters from control muscles were significantly larger than for the reinnervated muscles.



Figs. 1–4. Photographs of teased, whole-mount, silver-impregnated preparations of muscle spindles of the adult cat soleus muscle following cross-reinnervation. Scale bar = 50 μm.

Fig. 1. Muscle spindle from cross-reinnervated muscle with equatorial region (eq-reg) devoid of sensory endings. The axon bundle (a) at the top of the figure ran parallel with the intrafusal fibers without making any contact. The largest axon visible was 3 μm in diameter.

Fig. 2. Another spindle from cross-reinnervated muscle with noninnervated equatorial region (eq-reg) of the intrafusal fiber. Nuclear bag and nuclear chain fibers can be seen. Again, there were axons that ran close to this spindle but stopped abruptly without entering the capsule and giving off any visible terminals (out of field of view).

Fig. 3. Cross-reinnervated muscle spindle showing parallel fine wavy terminals (t) given out by a medium-sized axon (a), 5 μm in diameter.

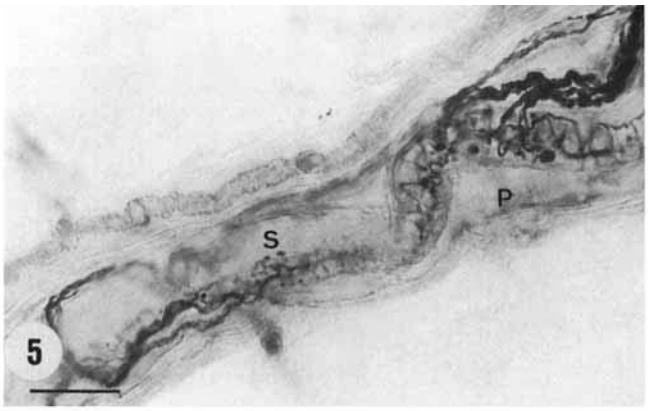
Fig. 4. Cross-reinnervated muscle spindle showing definite annulospiral endings (a-s) supplied by a reinnervating axon (a). In this spindle, there were parallel fine wavy terminals present as well, but they are not visible in this focal plane.

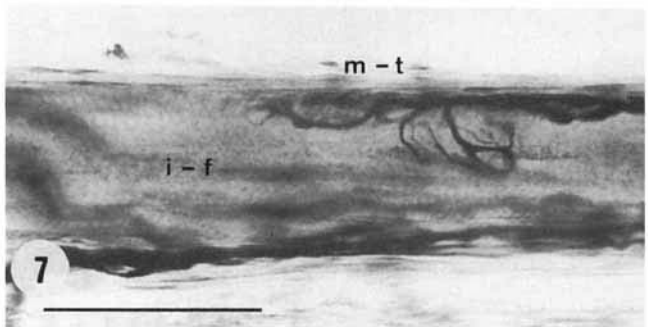
DISCUSSION

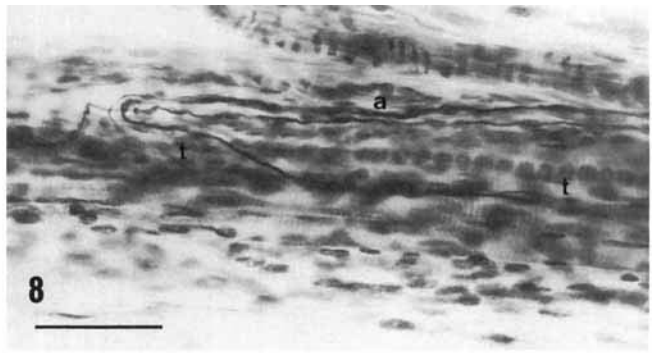
The main finding of this investigation is that the pattern of innervation of muscle receptors is extensively disrupted following reinnervation. Sometimes tendon organs and more rarely spindles reestablish a nearly normal innervation. At other times terminations constitute a network of fine branching endings from one or more parent axons with a diameter that is smaller than normal. The parent axon may be smaller because it is one of several sprouts that have arisen at the neuroma (Horch and Lisney, 1981) or an axon that has innervated the wrong receptor (Collins et al., 1986). Occasionally, no innervating axon could be detected at all. Instances of incomplete staining would only partially account for the large number of empty receptor capsules encountered in all experimental muscles. It appears that some targets were completely missed by the outgrowing nerve fibers. In this context it is of interest that Gregory et al. (1982) encountered many abnormal responses from sites located close to the entry point of the nerve into the muscle, whereas distal parts of the muscle appeared less densely innervated. It may be that some outgrowing afferent fibers on entering the muscle encounter receptor sites that are already occupied and are unable to seek out more distant sites because the neurotrophic signal is too weak.

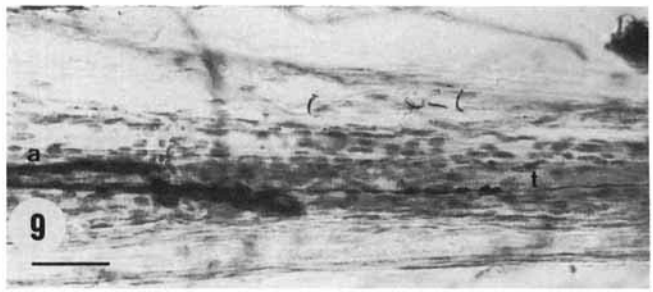
In their study of self-reinnervated muscle Collins et al. (1986) were able not only to characterize the responses of the receptors, but to determine whether the afferent fiber generated field potentials in the homonymous motoneuron pool. They concluded that reinnervation of muscle receptors following nerve section was probably a random process, in which some axons made inappropriate peripheral connections. For example, where the axon could be identified by its synaptic potential as having originally belonged to a muscle spindle, it was found innervating a tendon organ. The central connections of such afferents eventually became nonfunctional.

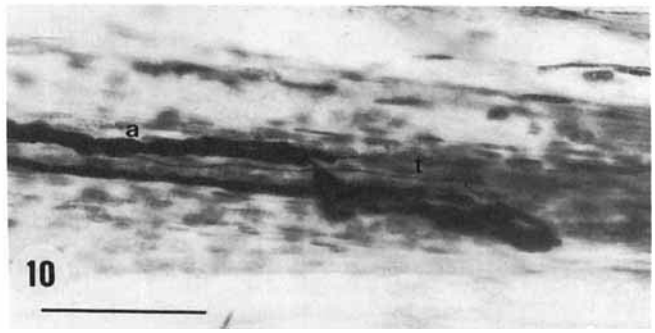
Our experiments had originally been carried out both on self-reinnervated and on cross-reinnervated muscles











Figs. 5–10. Photographs of teased, whole-mount, silver-impregnated preparations of muscle spindles of the adult cat soleus muscle. Figure 5 is from a control muscle, Figures 6–10 from self-reinnervated soleus muscle. Scale bar = 50 μm.

Fig. 5. Control soleus muscle. The sensory region of a normal spindle showing the typical annulospiral primary (P) and "flower-spray" secondary endings (S).

Fig. 6. Self-reinnervated muscle. Spindle showing a multitude of parallel fine wavy terminals (t).

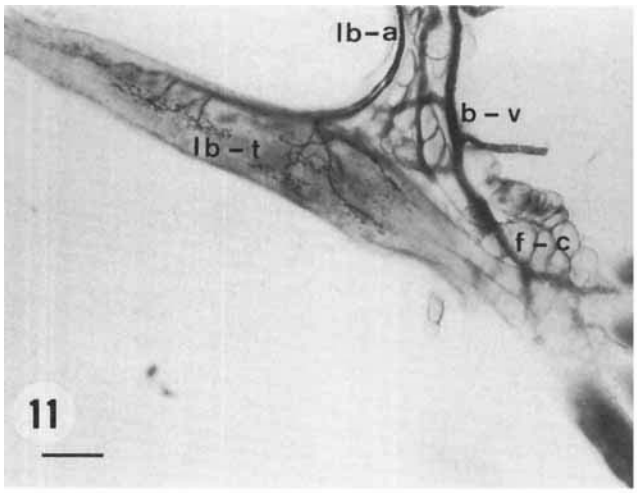
Fig. 7. A motor terminal of normal appearance (m-t) on an intrafusal fiber (i-f) from a spindle of a self-reinnervated muscle. The 6-μm-diameter axon terminates as numerous fine threads in the equatorial region (not shown) of this spindle.

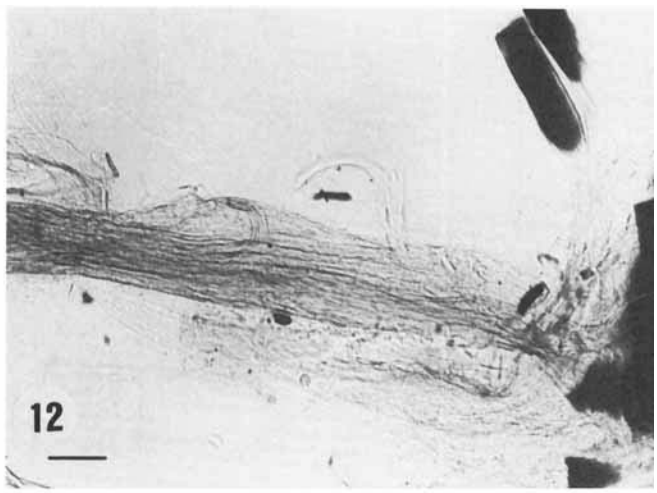
Fig. 8. Spindle of a self-reinnervated muscle with an axon (a) about 3 μm in diameter terminating as fine threads (t). No spirals or coils are visible.

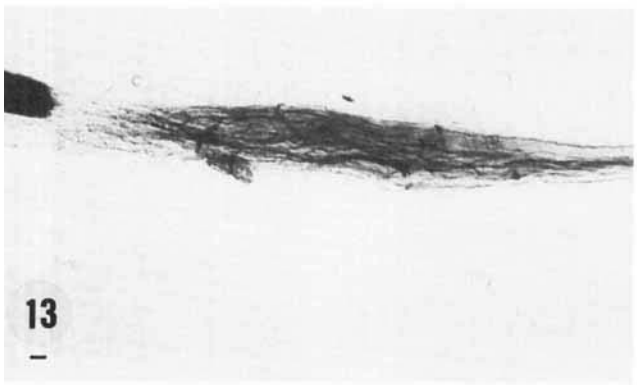
Figs. 9 and 10. Two magnifications of a spindle (higher magnification in Fig. 10) from a self-reinnervated muscle supplied by an axon (a) 4–5 μm in diameter that does not terminate as spirals or coils but tapers and ends abruptly as fine terminals (t). The nuclear bag and nuclear chain intrafusal fibers appear to be devoid of any sensory endings.

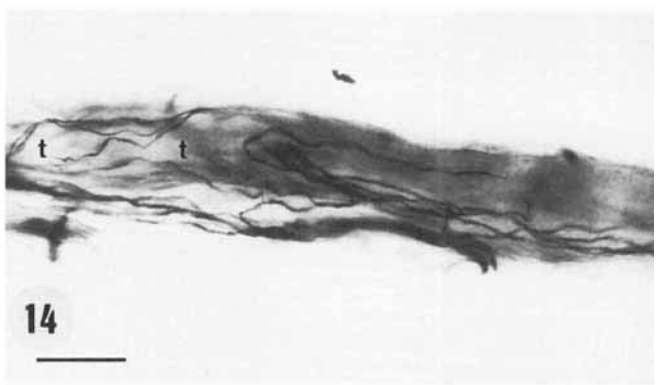
with the aim of detecting effects attributable to the foreigness of the nerve. Unfortunately, the sample size was too small for us to draw any conclusions on this point. The two self-reinnervated muscles yielded 41 identified receptors compared with 217 receptors from the eight cross-reinnervated muscles. Most of the receptors from the self-reinnervated muscles showed some form of abnormality. Because of the small sample, this cannot be interpreted as implying that the degree of abnormality was any different between the two preparations. Our earlier physiological studies had in fact suggested that responses recorded from self-reinnervated muscles were more nearly normal than those from cross-reinnervated muscles. Another point to consider is that if reinnervation following nerve section is random, this may overshadow influences attributable to the foreigness of the nerve. The fact that on many occasions in both self- and cross-reinnervated muscles the end organ was reinnervated by axon terminals that were grossly abnormal points to some form of interaction between the outgrowing axon and the innervated target. However, such communication does not seem to be able to prevent formation of inappropriate connections.

An important factor that may be considered to influence the pattern of reinnervation is the nature of the nerve injury itself. It is now well established that nerve









Figs. 11–14. Photographs of teased, whole-mount, silver-impregnated preparations of tendon organs from control (Fig. 11) and cross-reinnervated (Figs. 13, 14) soleus muscles of the adult cat. Scale bar = 50 μm.

Fig. 11. Control soleus muscle. A normal tendon organ showing afferent axon (Ib-a) and typical terminal (Ib-t) endings. The extrafusal fibers terminate on the capsule to the right. Blood vessels (b-v) and fat cells (f-c) appear along the left side of the axon.

Fig. 12. A tendon organ from a cross-reinnervated muscle devoid of any axon or ending. This "end-organ" was identified as a tendon organ on the basis of its location. It is shown connected "in series" with a fascicle of extrafusal muscle fibers that can be seen to the right.

Figs. 13 and 14. Two magnifications of a tendon organ from a cross-reinnervated muscle with an abnormal network of endings. Figure 14 is at a higher magnification to show more clearly the details of the extensively branching network of terminals (t). This tendon organ is also is "in series" with extrafusal fibers, which appear to the left.

crush or section produces some permanent disruption of the innervation of muscle. More specifically, muscle receptors retain identifiable structural abnormalities 50 weeks after nerve section (Banks et al., 1985). This finding agrees with the observations made here on preparations fixed 227–449 days postoperatively. It is necessary to take into account the length of the postoperative period, since there are likely to be many transient abnormalities accompanying the onset of reinnervation that are subsequently corrected. Furthermore, the nature of the injury, whether it is nerve crush or section, is important, since it is known to influence the subsequent degree of recovery (Brown and Butler, 1976). Finally, selective lesion of motor fibers impairs recovery of muscle spindles following nerve crush (Ip et al., 1977). It is therefore necessary to consider recovery processes in both afferent and motor fibers.

The experiments of Banks et al. (1984) have shown that when the nerve innervating the test muscle contains a majority of afferents of nonmuscle origin, some functional reinnervation of muscle receptors is still possible (see also Ip and Vrbova, 1983). They concluded that tendon organs and cutaneous and joint afferents were all capable of innervating spindles that were subsequently able to generate responses to muscle stretch. The EDL nerve is obviously better matched to the soleus muscle than is a skin nerve. It should be remembered, however, that EDL has 80% more group I and II afferents than soleus (Boyd and Davey, 1968). There are therefore too few end-organ targets for the number of innervating axons, which is likely to lead to examples of hyperneurotization (Barker and Boddy, 1980; Barker et al., 1985) and an increased incidence of free nerve endings in the muscle.

In agreement with the earlier conclusions of Ip and Vrbova (1973), Barker and Boddy (1980) found that after nerve crush the sensory innervation of muscle spindles was reestablished less successfully and more slowly than the motor innervation. Reinnervation of tendon organs led to the reestablishment of a majority of nearly normal

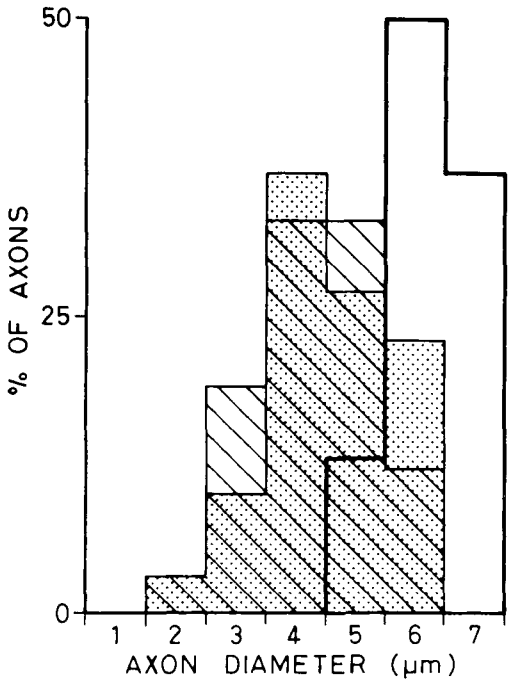


Fig. 15. Distribution histogram of axon diameters of muscle afferents from the cross-reinnervated (▨), self-reinnervated (▤), and control (□) muscles. Numbers of axons measured were 106 in cross-reinnervated, 30 in self-reinnervated, and 16 in control muscles. Many axons (40 in cross-reinnervated, 4 in self-reinnervated, and 5 in control muscles) going to receptors could not be measured either because they were hidden from view among several axons running in parallel or because only a very short segment of axon close to the receptor remained.

receptors although there remained many abnormal spindles. In this study we also observed many more normal tendon organs (38%) than normal spindles (3%) in the reinnervated muscles. On the other hand, in physiological experiments on receptors of cross-reinnervated muscles (Gregory et al., 1982), responses were obtained from fewer reconstituted tendon organs (7%) than spindle primary endings (16%) (see also Collins et al., 1986). It may well be, of course, that some of the tendon organs with an apparently normal structure were functionally abnormal. Similarly, it is possible that some of the spindles that gave apparently normal physiological responses had detectable structural abnormalities.

In a recent study Banks et al. (1985) examined the effects of nerve section on the structure and discharge properties of muscle receptors. They found that 25% of spindles and 45% of tendon organs recovered normal function. Such a high degree of recovery may be related to the surgical procedures used to repair the sectioned nerve. The cut nerve ends were carefully realigned and rejoined with epineural sutures. In the present study the cut ends were simply held together by ties attached to each nerve stump.

The original aim of our experiments on cross-reinnervated muscles was to try to establish whether the abnormal responses from muscle receptors might be a factor in the observed transformation of the contractile properties of the muscle. The fact that we encountered abnormal responses in self-reinnervated muscles as well, argues against a major role for muscle receptors in the transformation process. The recent observations of Luff et al. (1984) have confirmed that even after deafferentation transformation still takes place. In a related series of experiments Goldring et al. (1981) showed that in a soleus muscle innervated by both its own nerve and the nerve to flexor digitorum longus (FDL), stimulation of the FDL nerve produced a faster contraction than stimulation of the soleus nerve. They found that this difference persisted after deafferentation. It is reasonable to conclude that muscle afferents are probably not directly involved in the transformation process, although they may well play a contributory role. In this context it is of interest that Luff and Webb (1985) found that in freely moving cats the pattern of activity recorded from the soleus muscle cross-reinnervated by the EDL nerve was unlike that of either a normal soleus or normal EDL muscle. The observed differences were attributed to a combination of a lack of proprioceptive information and some degree of central remodeling.

In conclusion, we find extensive disruption of the normal pattern of innervation of muscle spindles and tendon organs following both self-reinnervation and cross-reinnervation of soleus with the EDL nerve. We suspect that some of the disruption and the accompanying establishment of abnormal afferent endings is attributable to the nature of the nerve injury and the method used to reconnect the cut nerves.

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