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Reprinted from
J. Neurophysiol., 1945, *8*: 173-194

SENSORY-MOTOR NERVE CROSSES IN THE RAT*

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(Received for publication February 26, 1945)

THE URGENCY of creating optimal conditions for nerve regeneration in man has renewed the interest in the fate of regenerated nerve fibers shunted into inappropriate pathways and to foreign terminations. In the regeneration of mixed nerves, a majority of fibers may suffer this fate. The following relations between a regenerating nerve fiber and its future destination have variously been suggested. (a) A nerve fiber of a given type might be attracted selectively toward a distal fiber stump of the corresponding type ("neurotropism"). (b) The fiber might approach all distal stumps indiscriminately, but be admitted only into a tube of the corresponding kind. (c) The fiber might enter tubes of all kinds, but the Schwann cells (Büngner's cords) in the tubes might have retained some of the original fiber specificity and might impede the growth of foreign axons. (d) The nerve fiber might grow down any sort of distal fiber stump but be barred from entering a strange peripheral organ by some selective repulsion. (e) The fiber might invade the peripheral tissue, but fail to form proper connections. (f) The fiber might form a structural connection capable of transmitting impulses but lack an essential physiological mediator of transmission. (g) The fiber might first form transmissive connections but, lacking functional activity, gradually be resorbed.

Of these suggestions many can now be discounted. "Neurotropism" (a) has been ruled out conclusively (39); the entry of fibers into strange tubes (b), their continued growth in these (c), and their spreading in the strange tissue at the end (d) have been demonstrated by Boeke (5) and others since; and the problem thus narrows down to a decision between points e, f, g. For practical purposes, we shall confine ourselves to the somatic motor and sensory fibers, which form the subject of this paper. Do proximal sensory fibers regenerating into a distal motor path establish transmissive connections with the muscle fibers to which they are led, and do motor fibers regenerating into a sensory path connect with end organs in the skin? If so, what happens subsequently to these functionally useless structures? If not, does the peripheral tissue at least derive some trophic benefit from their presence? Although the first experimental approach to these questions dates back more than a hundred years (3; for a review of the older literature, see 29), the various solutions offered in the past have remained equivocal, mainly because of inconclusive experimental conditions.

* This work was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the University of Chicago. It was also aided by the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

Langley and Anderson (22) connected the proximal stump of the sensory saphenous nerve to the distal sciatic in a single rabbit and reported that 13 months later electrical stimulation of the distal stump gave reflex responses but no direct contraction of the muscles supplied by the sciatic. The authors concluded "that the afferent fibers of the internal saphenous nerve had 'united' with the afferent but not with the efferent fibres of the sciatic" (p. 374). As Langley was an advocate of the "outgrowth" theory of nerve regeneration, the authors presumably meant to imply that sensory fibers grew only into sensory and avoided motor pathways. At any rate, they unmistakably ascribed the failure of this sensory-motor nerve cross to selectivity in regeneration, rather than in peripheral transmission.

Bethe (2), in repetition of earlier experiments by Schiff, cut and resutured dog sciatics, and after allowing sufficient time for regeneration, stimulated the spinal roots. As stimulation of the dorsal roots produced no muscle contractions, he concluded that sensory fibers proximal to the lesion had failed to "connect" with the distal stumps of motor fibers. Thus, he too, explained the results in terms of selective regeneration.

Boeke (5), however, proved histologically that in reciprocal crosses of sensory and motor nerves the proximal fibers of either type can grow down the distal stump of the other and form terminal connections with the foreign tissues to which they are thus led. The terminations of sensory fibers on muscle fibers resembled closely ordinary motor endings, but Boeke applied no stimulation tests to see whether they could transmit impulses. He did intimate restoration of trophic control by heterologous fibers.

Weiss (29, 30), working with toads, implanted the cut ends of sensory nerves directly into denervated muscles. In order to procure purely sensory nerves, he cut the motor roots of the lumbo-sacral segments, translocated the spinal ganglia with their attached nerves into the body cavity and inserted either the free end of the dorsal root or the distal cut surface of the whole "de-efferented" spinal nerve into a muscle transplanted within reach. Histological studies showed that the sensory fibers had spread throughout the muscle and wound themselves around the muscle fibers ending in highly atypical arborisations. When stimulated electrically, these nerves gave results different from those observed after insertion of motor nerves into muscles. Indirect excitability was found only in less than half of the operated specimens, and where it did appear, its onset came about three times as late as after motor nerve implantation. While the negative cases were taken as evidence of an intrinsic difference between motor and sensory fibers, the positive cases were considered as proof that these differences rendered the innervation of muscle by sensory fibers merely more difficult, but not altogether impossible.

All of these past attempts to settle the question are open to criticism. Technical failure of the nerve union could be suspected as a cause of some negative results, while positive results could be ascribed to the intrusion of stray fibers of uncontrollable origin into the crossed nerves through the loop-

holes of the suture line. Since these imperfections of technique can be eliminated by the method of arterial splicing recently developed by Weiss (33), we have reinvestigated the problem with this new technique under precautions not observed in previous work.

METHODS

The experiments consisted of reciprocal crossing of sensory and motor nerves. The combination of a proximal sensory with a distal motor stump is easy to test physiologically as ascending transmission evokes a reflex response, while descending transmission, if present, should register as a muscle contraction. Physiological tests of the reciprocal cross—proximal motor to distal sensory nerve—would require action potential registration. For technical reasons, we have thus far only examined the former type.

White rats (100–200 g) were used. The femoral nerve offers a favorable preparation, as it splits into two divisions of predominantly sensory and motor content, respectively. The operations are explained in the diagram, Fig. 1. The femoral nerve generally receives its fibers from segments L2, L3 and L4 (Fig. 1A). In the thigh it divides into two trunks M and N (Fig. 1B), the former being the motor nerve to the m. quadriceps (ca. 950 medullated fibers), while the latter consists of three smaller components, namely, two sensory fascicles constituting the n. saphenous (S), containing ca. 1700 medullated fibers, and the motor nerve to the m. pectineus (P), with ca. 200 medullated fibers.*

Crosses were effected between the quadriceps nerve (M) as motor nerve and the n. saphenous (S) as the sensory nerve. The presence of a few proprioceptive fibers in the former is of no practical consequence in view of its otherwise purely motor content. The saphenous nerve appears to be purely sensory in physiological regards, both branches innervating skin of the mesial aspect of the limb, one terminating in the thigh, the other in the leg and foot. Electrical stimulation has never produced any muscle contraction. A histological study three days after ventral root section, however, revealed the presence in the normal saphenous nerve of a small number of degenerated myelinated fibers. This proves that the nerve normally contains a few aberrant motor fibers with no functional connections. It likewise illustrates the fallibility of judging the content of a nerve by purely physiological tests. Depending on whether the motor pectineus branch, which runs for a considerable distance in a common epineurium with the saphenous, is excluded or included in the operation, we tap either an almost pure sensory or a mixed nerve source; in the latter case the motor and sensory components are sharply segregated.

Crossing the proximal stump of the saphenous (Sp) to the distal quadriceps stump (Md), forces a practically homogeneous sensory fiber population into the quadriceps muscle (Fig. 1E). If the pectineus nerve is left with the saphenous and their proximal stumps (Sp, Pp) are jointly connected to the distal quadriceps nerve (Md), we place motor fibers and sensory fibers in competition for reinnervation of the muscle (Fig. 1C). In order to determine the part played under these conditions by the sensory fibers, it was necessary to eliminate the motor fibers again after regeneration had occurred. This was done in a second operation, 3 months later, in which the proximal pectineus branch (Pp) was cut proximal to the nerve union. The cut end was tightly sealed with methyl methacrylate to preclude renewed regeneration (Fig. 1D). Various periods of time (3 days to 5 months) were then allowed before stimulation tests and biopsies were made. This interval was to provide for the degeneration of the severed motor fibers and the atrophy of muscle fibers formerly connected with them.

Finally, for a more radical elimination of all motor supply from the test area, the femoral nerve plexus of some animals was "de-efferented" five to six days prior to biopsy (Fig. 1A). This was done by dorso-lateral exposure of the entire origin of the femoral nerve, permitting easy identification of its roots. Transection of the ventral roots L2, L3 and L4

* This nerve distribution, consistently observed in the rat strain used by us, differs from the illustrations presented by Greene (13) for the Wistar strain. According to the latter, the motor nerve to the m. pectineus leaves the common femoral nerve jointly with the n. iliacus far proximal to the point where the saphenous and quadriceps nerves separate, while in our specimens, it remains joined to the n. saphenous considerably beyond this point.

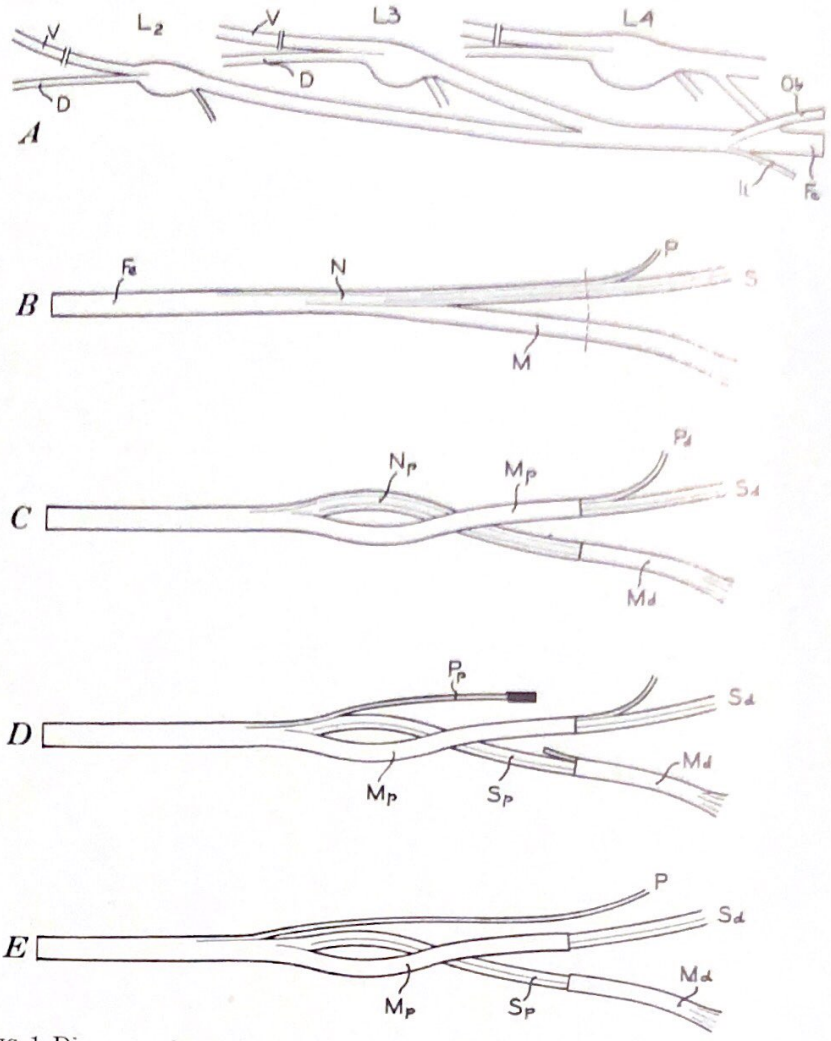

FIG. 1. Diagrams of operations

A. Roots of femoral plexus, indicating levels of transection in "de-efferentation."

B. Normal branches of femoral nerve.

C. Cross of common stump containing saphenous and pectineus nerves to quadriceps stump.

D. Same operation as C, followed later by proximal resection of pectineus nerve.

E. Crossing of saphenous stump to quadriceps stump.

Symbols:

D, dorsal root; Fe, femoral nerve; Il, iliacus nerve; M, quadriceps nerve; N, common trunk of saphenous and pectineus nerves; Ob, obturator nerve; P, pectineus nerve; S, saphenous nerve; V, ventral root. Small letters p and d refer to proximal and distal stumps, respectively.

was performed intradurally, 4–6 mm. central to the union of the roots; the dura over the sensory ganglia was not removed. The completeness of the operation was always checked at autopsy and, in most instances, histologically.

Two types of control operations were performed. In one, the saphenous and quadriceps nerves were simply cut and reconnected each to its own stump without crossing as a test of normal recovery. In the second, the quadriceps nerve was cut and capped with plastic, to determine the effect of chronic denervation. Determinations of the weights and fiber sizes of the denervated muscles were then made after from one to four months.

All nerve unions were effected without the use of sutures by inserting and opposing the cut ends in fitting sleeves of artery, as described previously (33). Freshly excised segments of carotid artery from young rats (40–60 g) insured proper fit for the particular nerves and sizes of animals used in the experiments. A sleeve fitting too loosely would permit fiber escape and penetration of extraneous stray fibers, while too tight a fit might produce pressure-block (37). For reasons outlined previously (35, 38), a properly executed arterial splice insures the most direct, unconfused and straight transit of regenerating nerve fibers from the proximal to the distal stump. This technique, therefore, precludes the uncontrollable intrusion of fibers of undetermined origin into the crossed nerves at the suture site—an unavoidable hazard of all ordinary suture methods.

All stimulation tests were made under light nembutal anaesthesia with an induction coil and silver wire electrodes. All necessary precautions against escape currents were observed. The nerves were stimulated at various levels proximal and distal to the cross union. Stronger stimuli are required in the regenerated portion because it contains more connective tissue. After recording the sensory responses, the nerves were cut at a level 10–15 mm. proximal to the splice, to make certain that contractions observed in the test muscle were produced directly through the stimulated nerve, and not reflexly through collateral motor fibers that might have entered the muscle from the vicinity.

In some of the cases with motor response, the maximum isometric tensions developed by the experimental and control (contralateral) muscles were measured kymographically. The shape and arrangement of the quadriceps muscle (scattered origins; diverse orientation of fibers) make it impossible to transmit the tension of the entire muscle to the recorder; some of it is always dissipated in shortening the more peripheral fibers. Accordingly a more compact experimental muscle, even though smaller, can sometimes be recorded more effectively than a muscle of normal dimensions.

After the tests, the experimental and control muscles were excised and weighed. Samples were either fixed, sectioned and stained (silver impregnation according to Bodian or Bielschowsky, followed by Mallory Triple Azan) or dissociated in nitric acid and mounted in glycerin without staining. Muscle fiber diameters recorded in the tables below refer to the size range within which the majority of the fiber population of a given muscle lies. A single range is typical of normal or fully recovered muscle, while experimental muscles mostly show two distinct ranges, a smaller one representing degenerated fibers, and a larger one of undegenerated or recovering, though atrophic, fibers.

Nerve splices and spinal roots were preserved for myelin (Mahon, osmic acid) or axon (Bodian) staining.

Results

Gross examination at biopsy revealed in most cases firm nerve union with no constriction or adhesions at the site of the splice. Microscopic study confirmed the pictures previously described as characteristic of arterial sleeve splicing (33, 38). In technically adequate unions, the fibers of the proximal stump regenerated straight and smoothly across the gap into and down the distal stump (Fig. 2). There was no fiber exchange between the nerve and its surroundings and no anastomosis between the two operated nerves. The sleeves had achieved the desired insulation of the suture sites.

1. *Control experiments*

Weight loss and degenerative changes were recorded for five muscles which had been totally denervated for from one to five months (Table 1).

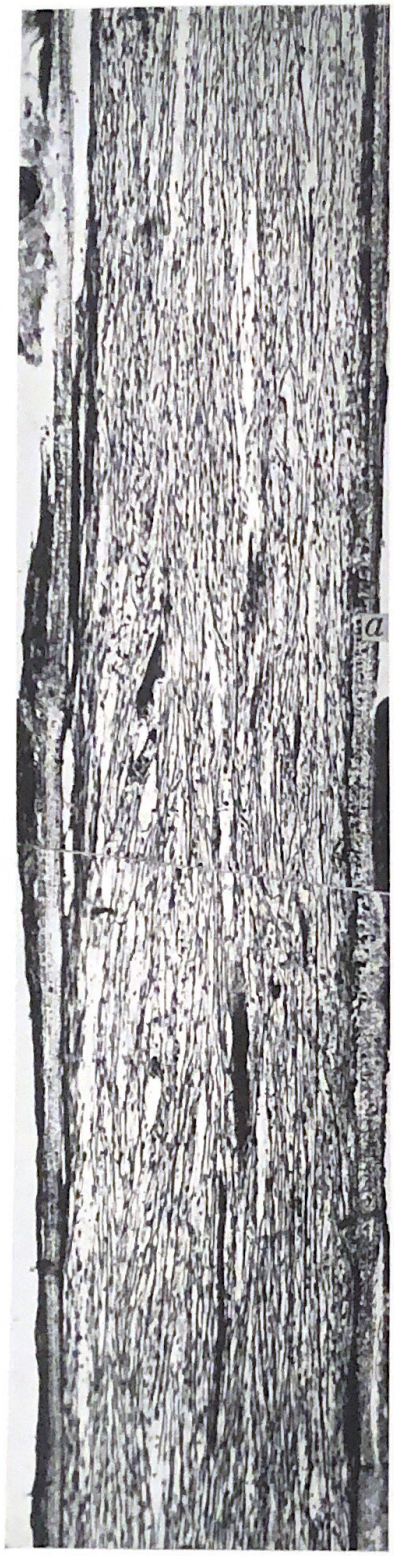

FIG. 2. Transition zone between the former proximal stump of the sensory saphenous nerve (bottom) and distal stump of the motor quadriceps nerve (top) inside arterial sleeve (a), 3 months p. op.—Bodian impregnation—ca. 100 ×.

The lower edge of the picture coincides with the level of the original cut surface of the proximal stump. There is no longer any demarcation between the former stumps.

The muscles lost weight at a steadily declining rate until, at the end of the third month, a nearly stationary level was reached in the neighborhood of 14 per cent. Since this value corresponds to the approximate proportion of connective tissue and vessels in muscle (21), we may consider three months a sufficient period for the practically complete disappearance of muscle substance after denervation. In the histological picture, the weight loss was reflected in a reduction of muscle fiber diameter and an increasing number of degenerating fibers. After two months, most of the fibers showed degenerative changes (vacuolation; loss of striation), although there were still several of rather normal appearance except for size. Muscles denervated for four months still contained a few relatively large and undegenerated fibers (8–20 micra), but the great majority of fibers were of minimal size (2–6 micra) and degenerated. This situation contrasts with that in the experimental muscles where cytologically normal fibers were found in considerable numbers, even in the oldest cases.

The recovery of quadriceps muscles, reinnervated by their own motor fibers, was studied in five animals kept two to six months after the transection and reunion of the nerve (Table 2). Almost complete return of extensor function in the leg was observed in every animal. Although the muscle in the shortest case (R 366) weighed

only 44 per cent of its control, in the older cases, a progressive increase in weight, reaching 88 per cent of the control after six months, was noted.** Histological preparations of the two oldest cases indicated that the great majority of fibers had been reinnervated, as fibers of the degenerated type were present only sporadically. The diameter of the largest fibers in these muscles

Table 1. Weight loss and decrease of fiber size in totally denervated muscle

Case	Duration months	Muscle weight in per cent of contralateral control	Muscle fiber diameter μ	
			control	experimental
R 375	1	32	30–50	25–40 10–15
R 374	2	18	38–45	15–25 5–10
R 369	2	19	25–45	12–20 5–8
R 362	3	13.5		
R 338	4	10	50–70*	30–40* 5–12
R 368	4	19	75–100*	15–25* 4–10

* These values were obtained from muscle fibers dissociated in nitric acid, which caused swelling to about twice the size of fibers fixed in Bouin's. The absolute diameter measurements are therefore too high, but have relative validity within the group. For comparison with muscles fixed in Bouin's, these values should be divided by two, which correction was made throughout the text.

was 87 per cent of that of the largest fibers of the contralateral control. The correspondence between this value and the degree of muscle weight recovery (Table 2), in conjunction with the close correspondence between weight and strength recovery described by Hines, Thomson and Lazere (18), might suggest a correlation between degree of recovered strength and maximum fiber size. However, other observations do not seem to support such a strict correlation, and further experiments on the subject are needed.

2. *Cross of proximal sensory stump to distal motor stump*

This series (Fig. 1E) includes three muscles (Table 3), biopsied three

** The failure of rat muscles to recover nearly full weight after nerve splicing has been noted in previous series of experiments. Hines, Thomson and Lazere (18) likewise report only 88 per cent of weight recovery after nerve crushing (84 days p. op.). This contrasts with values of up to 100 per cent observed in cats and monkeys after comparable operations (34).

Table 2. Recovery of weight and fiber size in muscle after reinnervation by its own nerve

Case	Duration months	Muscle weight in per cent of control	Muscle fiber diameter μ	
			control	experimental
R 366	2	44		
R 372	3	67		
R 360	3	74		
R 361	5.5	84	100–150*	70–130*
R 370	6	88	85–120*	75–95*

* See Footnote to Table 1.

months after connecting the sensory saphenous to the motor quadriceps stump.

In one case (R 402), no contraction of the muscle could be obtained upon nerve stimulation. Histological study showed this nerve cross to be exceptional because the distal stump had retracted from the sleeve. In the remaining two cases (R 400 and 401), stimulation of the nerve produced definite contractions of the muscle. However, these contractions were not mediated

Table 3. Cross of proximal saphenous nerve to to distal quadriceps nerve

Case	Duration months	Muscle response after electrical stimulation of:				Muscle weight in per cent of control	Muscle fiber diameter μ	
		Peripheral nerve (Sp or Md)*	Motor roots	Sensory roots	Peripheral nerve (Sp or Md)* after deefferentation		Control	Experimental
R 400	3	++	++	−		19	70–105**	50–80** 14–20
R 401	3	+			−	26		
R 402	3	−				12	90–120**	30–40** 8–15

* Symbols refer to Figure 1E.
++ vigorous contraction.
+ weak contraction.
− no contraction.
** fibers treated in nitric acid; see Footnote to Table 1.

by the regenerated sensory fibers, but by unsuspected stray motor fibers present in the stimulated nerve, as revealed by the following tests.

In case R 400, a vigorous response of the m. quadriceps to stimulation of the proximal n. saphenous was recorded. Thereupon the whole femoral plexus was dissected free, the roots were cut near the cord, and their distal stumps were stimulated separately. While contractions of the same magnitude as before were obtained from the ventral roots, stimuli applied to the dorsal roots remained wholly ineffective. The other case (R 401) gave a weak, but unmistakable, muscle response to stimulation of the saphenous nerve, when first exposed. The ventral roots of the femoral plexus were then cut and five days were allowed for motor fiber degeneration. Subsequent stimulation of the n. saphenous no longer produced any contraction in the muscle, while it continued to yield a strong reflex response of the animal, proving that the root operation had not damaged the sensory fibers of the nerve.

Two facts become clear from these observations. First, in no case have the sensory fibers formed transmissive connections with the muscle fibers. Second, whatever motor connections there were had been effected by ventral root fibers present in the saphenous nerve.

Histologic study showed that the sensory fibers had regenerated through the motor stump in perfectly normal fashion. Myelinization was good. The regenerated fibers had pervaded the muscle with extensive arborizations which would have provided ample opportunity for the establishment of myo-neural junctions. The only fibers that did form transmissive connections were the few ventral root fibers mentioned before; the sections showed them in degeneration owing to the recent root section. The muscles weighed from 12 to 26 per cent of the controls (Table 3). This reduction is of the same order as that of muscles denervated for a similar period (Table 1). Histologically, they consisted for the most part of atrophied and degenerated fibers of the small size class, in addition to a comparatively few large and healthy fibers. The latter account for the contractions observed in the physiological tests.

3. *Cross of proximal sensory and motor stump to distal motor stump*

In twelve cases, the proximal saphenous and pectineus nerves were joined to the distal quadriceps stump (Fig. 1C). The proportion of sensory to motor fibers in the proximal stump is nearly 9:1. While both components are strictly segregated at the source, they could be expected to intermingle somewhat during the transition from the proximal to the distal stump.

Nine cases were biopsied from two to five months after nerve crossing (Table 4). In every preparation, stimulation of the nerve, either proximal or distal to the cross union, resulted in a vigorous contraction of the quadriceps muscle. Kymographic records were taken from five of these muscles and yielded absolute tensions ranging from 75–100 per cent of the control for direct tetanic stimulation, and 57–88 per cent for indirect tetanic stimula-

tion. However, since the anatomical arrangement of the quadriceps muscle is such that the amount of tension lost in isometric recording (see "Methods") is the less, the smaller the muscle, the values of the experimental muscles, which are undersized (see below), are presumably too high in proportion to the controls.

In two further cases (R 301, R 340), in which stimulation of the nerve,

Table 4. Cross of proximal saphenous sensory and pectineus motor nerve to distal quadriceps nerve

Case	Duration months	Muscle response after electrical stimulation of:				Muscle weight in per cent of control	Muscle fiber diameter μ	
		Peripheral nerve (Np or Md)*	Ventral roots	Dorsal roots	Peripheral nerve (Np or Md)* after deefferentation		Control	Experimental
R230-2	2	++				69		
R277	2.5	++				41		
R279	3	++				35	35–55	25–35 3–10
R303	3	++				40	40–50	15–35 3–10
R251-2	3	++				43	30–50	14–22 3–8
R333	3.5	++				69		
R332	4	++				56	75–85**	50–70**
R339	4	++				33	60–80**	45–60**
R316	5	++						
R343	5	++	++	−		32	60–100**	45–80** 10–18
R301	6	++			−	27		
R340	7	++			−	52	25–45	18–35 4–11

* Symbols refer to Fig. 1C.
** Fibers treated in nitric acid; see Footnote to Table 1.

six and seven months postoperative, respectively, gave strong muscle contractions, the ventral roots of the femoral plexus were cut and the motor fibers allowed to degenerate for five and six days, respectively. Continuous fibrillation was observed in both muscles. Stimulation of the nerves no longer yielded any response of the muscles, although the latter responded promptly to direct stimulation (Table 4). Nerve stimulation, both proximal and distal to the union still produced marked reflex responses, and later histological study confirmed the fact that the sensory fibers had regenerated to the muscle and had not been damaged in the ventral root operation (Fig. 3).

In a final case (R 343) the entire femoral plexus was exposed and its roots were stimulated separately. Contraction of the quadriceps muscle could be

elicited by stimulation of the nerve or its ventral roots, but never from the dorsal roots.

The weights of the experimental muscles varied, without apparent relation to the length of time of recovery, from 27 to 69 per cent of their controls (Table 4, column 7). Most histological samples of these muscles (Fig. 4) showed muscle fibers of two distinct size classes, obviously correlating

FIG. 3. Case R 301.—Proximal pectineus (p) and saphenous (s) nerves, both of which had been united to the distal quadriceps nerve 6 months previously (series III), 5 days after ventral root section. Bodian impregnation—220 ×.

The motor pectineus nerve contains only an occasional fiber (proprioceptive or in delayed degeneration), while the fibers of the sensory saphenous nerve are intact. The degenerated ventral root fibers in the latter are too scarce to be distinguishable in the picture.

with whether or not they had received innervation (Table 4, column 9). The larger fibers ranged in diameter from 14 to 40 μ (47 to 80 per cent of the controls) and showed normal cytological details. Those of the second group were extremely atrophic (3 to 11 μ), and most of them had undergone severe degenerative changes. They were irregularly swollen and vacuolated, most cross striations had disappeared, and the nuclei were in various stages of disintegration. Smaller and larger fibers were not interspersed indiscriminately, but in the main a major sector of the muscle was occupied by fibers of one class exclusively. Individual fascicles of the first and second order were mostly composed either of degenerated or recovery fibers only (Fig. 4). Samples taken from two muscles (R 332 and 339) were exceptional since they did not contain fibers of the small class. All fibers were healthy and relatively large (67 to 82 per cent of the controls). Sampling errors may ac-

count for this discrepancy but it is significant that these two muscles also gave the highest isometric tension values recorded.

These results prove that only those muscle fibers have recovered size and indirect excitability which had become connected to motor axons, while sensory fibers, though regenerating into the muscle, had remained wholly ineffective.

4. *Cross of proximal sensory and motor stump to distal motor stump, followed by secondary removal of motor part*

In this series, the initial operation was the same as in the preceding series (Fig. 1C). Three months later the motor pectineus nerve (Pp) was severed proximally and its cut surface was capped with resin plastic to prevent re-

Table 5. Cross of proximal saphenous and pectineus nerves to distal quadriceps nerve, followed three months later by removal of pectineus nerve

Case	Time since removal of pectineus nerve	Muscle response after electrical stimulation of				Muscle weight in per cent of control	Muscle fiber diameter μ	
		Peripheral nerve (Sp or Md)*	Ventral roots	Dorsal roots	Peripheral nerve (Sp or Md)* after deefferentation		Control	Experimental
R 335	3 days	+				57		
R 337	10 days	++				35	80–90**	50–65**
								15–30
R 278	3 wks.	+				18	70–100**	45–70**
								5–25
R 326	3 wks.	++				39		
R 328	6 wks.	++	++	–		19	70–90**	35–55**
								5–15
R 327	2 mos.	+			–	14	70–100**	15–35**
								5–15
R 329	5 mos.	++			–	22		

* Symbols refer to Fig. 1D.
** Fibers treated in nitric acid; see Footnote to Table 1.

generation (Fig. 1D). The cap remained in place, effectively blocking the outgrowth of fibers. Seven cases of this type were studied three days to five months after the second operation (Table 5). Extension of the operated leg during normal activity of the animal was not observed, but in all cases, electrical stimulation of the nerve produced contraction of the quadriceps muscle. In three cases (R 335, R 278 and R 327), 3 days, 3 weeks and 2 months, respectively, after pectineus transection, the contractions after indirect stimulation were too weak to be recorded kymographically. Yet, the first of these muscles gave a strong contraction on direct stimulation, commensurable to its mass, which was 57 per cent of the control. The discrepancy between di-

rect and indirect stimulation is accounted for by the short time (3 days) which elapsed after cutting the pectineus nerve. In the other two cases of progressively longer duration, the muscle weights had dropped to 18 and 14 per cent of the control values and the isometric tensions on direct stimulation were correspondingly low. Evidently, therefore, these three muscles agree in that they had received only a few and physiologically barely demonstrable motor connections through the saphenous nerve.

In the four remaining cases, the residual muscular response was appreciable. However, these contractions were not produced by the sensory fibers, but by aberrant motor fibers running in the saphenous nerve. This was proved by tracing the origin of the functional fibers to the roots in two cases which had registered relatively strong muscle contractions. In one of these (R 328), the roots of the femoral plexus were exposed and stimulated separately. Muscle contraction could be obtained only from the ventral roots. In the other case (R 329), the ventral roots were cut: stimulation of the saphenous six days later no longer produced contraction, although the muscle still responded strongly to direct stimuli. Subsequent histological study proved that the ventral roots had completely degenerated. The saphenous nerve also contained a small number of fibers in the process of degeneration amidst the intact sensory fibers. The normal occurrence of a few ventral root fibers in the saphenous nerve has been reported above. This completes the evidence that all responses previously obtained in the muscles concerned had been mediated through these motor fibers, and none through the sensory fibers, although both types had invaded the muscle.

Muscles in this group had thus been neurotized from three different sources: (a) the mass of sensory fibers of the saphenous (Sp), which have failed to make transmissive connections; (b) the motor fibers of the pectineus (Pp), which had been removed again three days to five months prior to examination; (c) a few motor fibers contained in the saphenous nerve proper which persisted after pectineus transection, but degenerated after ventral root section. This situation is well reflected in the muscle weights (Table 5). During the three month period between the nerve cross and the renewed section of the pectineus, muscle weight had recovered to at least 60 per cent of normal (case R 335; 57 per cent was recovered, but allowance must be made for atrophy during the 3 days since nerve section). This compares with about 70 per cent recovery three months after re-innervation of the muscle by its own nerve (Table 2). Ten days after transection of fiber group (b), muscle weight has declined to 35 per cent (R 337), and after three weeks, most of the fibers formerly innervated by (b) have atrophied. The residual weight of the muscle is composed of degenerated fibers, connective tissue, and those healthy functional fibers innervated from source (c). Except for one case (R 326), this residual weight (average of 4 cases: 18 per cent of control) is not much above the range of muscles completely denervated for comparable lengths of time (Table 1), indicating the relatively small size

of contingent (c). Obviously, the presence of non-transmissive sensory nerve fibers in the muscle has had no effect on its trophic state.

5. *Histological observations*

A microscopic study of the nerve unions revealed no difference in the passage and penetration of regenerating nerve fibers into the peripheral stump, whether in homologous or heterologous combinations (Fig. 2). The sensory fiber masses had filled the distal quadriceps nerve stump and thus entered the quadriceps muscle through the old motor point. The main bundle then branched into smaller groups of fibers which spread throughout the muscle; from these smaller branches a few fibers at a time would detach themselves and pursue branching and frequently tortuous courses among the individual muscle fibers. The surface contact between these sensory fibers and the sarcolemma appears in microscopic observation to be as close as that of motor fibers. It was not possible, however, to ascertain finer details of the relationship between the sensory nerve fibers and the muscle fibers, and the manner in which the former terminate remains to be determined. Elaborate nerve endings were absent, but this could be attributed to incomplete staining. In no case was there any sign of resorption of the functionally useless fibers as late as eight months after the operation.

Discussion

The reported experiments have decided the alternatives listed in the introduction in favor of either point (e) or (f): sensory fibers grow down motor nerves, pervade the muscle, but remain incapable of transmitting impulses to the muscle fibers upon electrical stimulation. Technical imperfections inherent in earlier similar work have been overcome by two measures—combined physiological and histological checks, and hermetic shielding of the suture lines. Arterial sleeve splicing has been fully effective in preventing the undesirable ingrowth into the nerve junction of fibers from other than the designated sources.

Histologically, the regeneration of sensory fibers into motor nerves did not differ perceptibly from normal nerve regeneration (Fig. 2). We have been unable to confirm Boeke's conclusion (5, p. 53), that the outgrowth of sensory nerves into motor stumps occurs less smoothly than that into homologous stumps. Perhaps in his cases the stumps were not properly matched. Confusion of nerve fibers at the transition from the proximal to the distal stump is a result of improper technique (35), irrespective of the nature of the stumps. With proper treatment, sensory fibers pass on into a motor stump straight and unimpeded and grow down its length into the muscle.

After arriving in the muscle, the fibers follow the gross pattern of the motor nerve. Terminal branches are abundant and enter into microscopically close contact with the muscle fibers, around which they coil themselves. If neuro-muscular transmission were simply a matter of a gross electrical change along the contact surface, the total surface of these end branches

would have been large enough to give a potent discharge. Yet, no sign of contraction was ever observed upon maximal stimulation of the nerve. This indicates that subtler relations than mere surface contact are required to constitute a functional myo-neural junction.

The failure of sensory fibers to excite muscle must be attributed either to lack of proper end connection or lack of a physiological mediator. Dale (6) suggested the latter alternative. Sensory nerves have actually been shown to contain much less acetylcholine than motor nerves (24, 25, 11). However, while demonstrating the biochemical differential between the two kinds of nerves, this fact of itself is not sufficient to disprove the alternative of inadequate structural connections.* The possibility of a structural non-union between sensory fiber and muscle fiber because of incompatibility of the respective protoplasms is suggested by developmental considerations.

During the developmental phase, motor and sensory fibers demonstrate their constitutional differential by the fact that they can discriminate their respective pathways (16, 26). In consideration of the related tendency of fibers of either type to adhere selectively to fibers of their own kind ("selective fasciculation"), Weiss (32) has developed the concept that intimacy of contact between a nerve fiber and its surroundings is determined by specific affinities between the molecular populations on either side of the boundary membrane. A scheme to explain selective affinities between contiguous tissue elements in terms of correspondences or conformances of the molecular configurations along the contact surfaces has been presented on an earlier occasion (32). Holtfreter (19, 20) has produced striking examples of such selective affinities in embryonic development. Perhaps the establishment of a transmissive nerve connection is subject to a similar condition, namely, conformance between molecular species in the nerve fiber and its end organ. If such conformance existed between motor fiber and muscle fiber on the one hand, and between sensory fiber and sense organ on the other, but not crosswise, heterologous combinations would be incompatible. Failure of the nerve and end organ protoplasms to match might easily preclude the establishment of certain physical prerequisites of impulse transmission (intimacy of contact; molecular orientation; etc.), without our being able to tell a transmissive from a non-transmissive ending under the microscope. Actually, Boeke (5) has found no crucial microscopic difference in the manner in which motor and sensory fibers connect with muscle fibers. Gutmann and Young (15) have recently expressed an opinion on the fine-structural basis of nerve-muscle connections along lines similar to those presented above.

Muscles of series III and IV, innervated from both sensory and motor sources, offer several points of interest. Proximal stumps which contained both the saphenous and the pectineus nerve furnished the quadriceps muscle

* We have since repeated the experiment with an animal eserinized during the stimulation test (dosage: beginning at 2 mg. and rising to 4 mg. per kg. body weight). The resulting accumulation of acetylcholine did not, however, affect the result: tetanic stimulation of the sensory-motor nerve cross (extended for 30 minutes in series of 15 seconds each) continued to fail to yield muscle contraction.

with a fiber population in which the motor fibers were outnumbered by the sensory fibers in a ratio of nearly 9:1. Yet, this small motor contingent was capable of innervating the majority of the muscle fibers. This can be deduced from the recovered muscle weights (and the less reliable tension records) which in the best cases (R 230–2, R 333; Table 4) amounted to 69 per cent of the controls. This is almost as good a recovery as that following reinnervation of a muscle by its own nerve with five times as many motor fibers after comparable periods (Table 2). In other cases, the recovery was less (Table 4), but still out of proportion with the size of the motor contingent. The weight of a muscle is a function of both the number and the size of its fibers. Although we have recently discovered that innervated fibers in partially denervated muscles may hypertrophy beyond their normal size, there has been too little microscopic evidence of this phenomenon in the present cases to affect our deduction that larger muscle weights are chiefly due to greater numbers, rather than larger size, of innervated fibers. We arrive thus at the following conclusions.

(1) The motor fibers have innervated more muscle fibers than they would have normally done in competition with larger numbers of their kind. This confirms the capacity of motoneurons to carry an extra load of muscle fibers (7, 31, 1, 4, 36).

(2) Contact of a muscle fiber with a sensory fiber does not render the former immune to impregnation by motor fibers, as innervation by a motor fiber would do (10, 12). Only transmissive connection protects a muscle fiber against "hyperneurotization."

(3) The great variability in the degree of reinnervation from an undersized motor source (Table 4) indicates that the amount of terminal branching is largely a matter of accident, as previously indicated by Weiss and Campbell (36). There is obviously no mechanism in operation by which the denervated muscle fibers would continue to call forth branching until saturation were reached.

Histologically, the partially reinnervated muscles reveal the presence of two classes of muscle fibers: small degenerated fibers and larger undegenerated ones, the latter evidently being those that had received motor connections. Large sectors of the muscle containing exclusively one kind were rather sharply set off against other sectors composed exclusively of the other type (Fig. 4). This observation indicates a tendency of the motor neurons to stick together. Further experiments on this subject are under way.

The facts just discussed are further stressed by muscles innervated solely from the n. saphenous (pectineus nerve either omitted originally or eliminated later). The few ventral root fibers present in the resulting nerve cross produced some mucle contractions. Weights of these muscles (Tables 3 and 5) ranged between 14 and 22 per cent of the controls (except for one aberrant case—R 326). These values are not significantly above those of completely denervated muscles (Table 1), indicating that only a relatively small quota of muscle fibers had become reinnervated. Thus, while motoneurons can

take on an extra territory of muscle, this capacity for expansion is definitely limited (compare also 1, 17, 36). The limit is presumably set by the average amount of terminal fiber branches a motoneuron can sustain (35). It need not be assumed that this number is affected by the state of the muscle. A given neuron would undergo a standard average amount of intramuscular

FIG. 4. Case R 340.—Cross section of quadriceps muscle re-innervated by pectineus and saphenous nerves (series III), 7 months p. op.—Mallory Azan—80×.

Note sharp demarcation between central sector containing large (innervated) muscle fibers and peripheral zone of small (degenerated) fibers.

branching. However, owing to the resistance of muscle fibers to the admission of more than one terminal, more branches would miss a chance of becoming connected in a densely neurotized muscle than in a scarcely neurotized one, where they meet less competition. The increase in territory occupied by each motoneuron in a partially denervated muscle could thus be explained simply by the fact that supernumerary fiber branches, present in limited numbers, have taken hold, without postulating any regulatory excess production of new branches in response to some hypothetical influence

of the denervated muscle portion. The latter possibility has not been excluded, but it finds no support in the present experiments. The scarcity of motor innervation in these cases also underlines the fact that the failure of sensory neurons to connect with muscle fibers is not attributable to some competitive suppression by motor fibers, as might well have been assumed for mixed regeneration.

In practical regards, it is evident that in any mixed nerve after transection and reunion, a large fraction of motor fibers will happen into sensory pathways and vice versa. This results in a double loss. Motor fibers getting into sensory nerves are irretrievably lost as source of muscle innervation, and sensory fibers regenerating into motor pathways occupy spaces that could otherwise be utilized by motor fibers. However, these losses can be compensated for in part by the reported capacity of each motor fiber to connect with a much larger number of muscle fibers than normally. There are also signs of hypertrophy in the active fibers of a partially denervated muscle, which are still under investigation. For a further discussion of the fluctuation between volume of regeneration and muscle recovery, see Weiss and Campbell (36).

According to our experiments, the trophic maintenance and restoration of a muscle fiber depends on its possessing transmissive connection with a nerve fiber. Sensory fibers, though present in abundance and in contact with the muscle fibers, neither delay nor prevent denervation atrophy and degeneration. They cannot substitute for that "trophic" action of the motoneuron which maintains some contractile condition even in disuse (28, 8, 9).

Our experiences with partially denervated muscles likewise show that, contrary to an earlier indication (36, p. 622), the passive exercise given to the denervated fibers by their innervated partners has no perceptible effect. It must be borne in mind, however, that this exercise consists of passive shortening, while beneficial effects have only been reported from passive lengthening (stretching) or active contraction by electric stimulation of the denervated fibers themselves (27, 14, 9).

Since our experiments have shown conclusively that sensory fibers cannot form transmissive connections with muscle, they supersede earlier contentions to the contrary (29, 30). Those earlier experiments were done with amphibians, but it is highly improbable that different vertebrates should behave differently in such fundamental matters. A much more likely explanation is that despite precautions a small number of stray motor fibers had made their way into the sensory nerves in those cases in which muscular responses were obtained. This interpretation agrees with the fact that connections were formed only in a minority of cases, and even there were tardy in developing.

Summary

The problem of the regeneration of sensory nerve fibers into motor stumps and muscles has been reexamined in rats, using arterial sleeves to effect the

crosses. This technique prevents the intrusion of stray fibers from undetermined sources into the cross.

The proximal nerve source consisted either of a practically pure sensory nerve (n. saphenous) or of the latter plus a smaller motor branch (n. pectineus). These stumps were in all cases connected to the distal stump of the motor quadriceps nerve.

The sensory fibers regenerated into and through the motor nerve with ease, passed through the motor point, and pervaded the muscle with a rich network of arborizations closely applied to the muscle fibers. They exhibited no sign of selectivity in their regeneration into heterologous channels.

After regeneration had occurred, electric stimulation of the crossed nerve in most cases yielded contraction of the quadriceps muscle. Stimulation of the dorsal and ventral roots separately, or of the peripheral nerve after degeneration of all motor fibers following ventral root section, however, proved that the observed muscle contractions were mediated only by ventral root fibers included in the cross. In no case had sensory fibers formed transmissive connections with muscle fibers.

Sensory fibers do not affect the trophic condition of the muscle in which they end. Muscle fibers surrounded by sensory fibers exclusively undergo the same degree of atrophy and degeneration as if no nerve fibers were present.

The failure of sensory fibers to transmit impulses to muscle can be ascribed either to lack of proper structural connection, because of biochemical incompatability between the respective nerve and muscle protoplasms, or to lack of a physiological impulse mediator.*

In no case was there any histological sign of resorption of the functionally useless sensory fibers in motor pathways as late as eight months after the operation.

Muscles with mixed reinnervation recover weight to a greater extent than in proportion to the ratio of motor to sensory fibers present. Evidently, each motor fiber takes on an excess load; but this expansiveness of the motor unit is limited.

Histologically, the innervated fiber groups are rather sharply set off against the degenerated, uninnervated, ones. This fact indicates a tendency of the motor nerve fibers to stay assembled.

Earlier reports of successful innervation of muscles by sensory units can presumably be attributed to unsuspected contaminations of the sensory nerve sources by stray motor fibers.

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* See, however, footnote on p. 187.

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