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SYNAPSE FORMATION IN INTACT INNERVATED CUTANEOUS-PECTORIS MUSCLES OF THE FROG FOLLOWING DENERVATION OF THE OPPOSITE MUSCLE

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SUMMARY

1. Denervation of one cutaneous-pectoris muscle of the frog induces the formation of new synapses in the intact innervated muscle on the opposite side. After crushing the motor nerve to the left muscle the incidence of polyneuronal innervation in the right intact muscle increased from an average normal value of 16% to an average value of 27% (Rotshenker & McMahan, 1976).

2. The formation of the new synapses in the intact muscle is independent of the presence of denervated muscle fibres or degenerating axons peripheral to the site of axotomy. After removing the left cutaneous-pectoris muscle, the proportion of polyneuronally innervated muscle fibres in the right intact muscle increased to an average value of 34%.

3. The number of new synapses formed in one muscle is dependent upon the type of the lesion to the motor nerve to the opposite muscle; 40% of the muscle fibres on the right side were found to be polyneuronally innervated after transecting the motor nerve on the opposite side, as compared to 27% after crushing it.

4. The delay with which new synapses are formed on the unoperated side is dependent upon the distance from the spinal cord of the axotomy. New synapses were detected 4–8 weeks after cutting the opposite nerve close to the muscle. Placing the site of axotomy close to the spinal cord shortened the delay and new synapses were detected as early as 9 days after the operation.

5. The stimulus for the formation of new synapses by an intact nerve is ineffective if the injured nerve on the contralateral side originates from distant segments of the spinal cord. The pattern of innervation in cutaneous-pectoris muscles was not altered following denervation of distant muscles in the hind limb.

6. These results suggest that the signal for sprouting and synapse formation may arise in the damaged nerve cells, central to the site of axotomy, and then be communicated transneuronally within the spinal cord to the intact motoneurones on the opposite side.

INTRODUCTION

Sprouting of intact nerve fibres and synapse formation constitute a general response to partial denervation in both central and peripheral nervous systems (for reviews

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see Edds, 1953; Guth, 1974; Harris, 1974; Stein, Rosen & Butters 1974; Cotman & Lynch, 1976; Gutmann, 1976; Purves, 1976*a*, 1977). For example, if a muscle is partially denervated, the remaining intact axons sprout and form synaptic connexions with the denervated muscle fibres (Exner, 1885; Edds, 1950, 1953; Hoffman, 1950; Guth, 1962; Brown & Ironton, 1978). Recently, however, it has been shown that sprouting and synapse formation are not restricted to denervated tissues. After crushing the motor nerve to one cutaneous-pectoris muscle of the frog, the motor nerve to the opposite muscle sprouts and forms additional synaptic connexions with intact and already innervated muscle fibres (Rotshenker & McMahan, 1976). Some of the questions that arise from this observation concern the mechanism that underlie the induction of sprouting of intact motoneurones and synapse formation: what is the source of the signal for sprouting and synapse formation and how does it reach the responding intact motor nerve cells?

The signal for sprouting could arise distal to the site of axotomy from the denervated target tissue such as denervated muscle fibres or from degenerating axons or both, and reach the opposite muscle by local diffusion or via the circulation. Such mechanisms have been shown to play a major role in the innervation of denervated iris tissue transplanted to the anterior chamber of a host eye. There, intact nerve fibres of the host iris sprouted and innervated the denervated iris (Olson & Malforms, 1970). Similarly, degenerating nerve fibres and denervated muscle fibres have been proposed to be the source of the signal for sprouting and synapse formation in partially denervated muscle (Weiss & Edds, 1946; van Harreveld, 1947; Hoffman & Springell, 1951; Edds, 1953; Brown & Ironton, 1977*a*, *b*).

Alternatively, the source of the signal for sprouting and synapse formation could be the injured nerve cells, central to the site of the lesion. In that event the signal could be communicated from the damaged nerve cells to the intact motoneurones in the spinal cord.

The present study was undertaken to find the site where the signal for sprouting and synapse formation arises from and by what pathway it reaches the intact motor nerve cells. The separation between the site of denervation (one cutaneous-pectoris muscle) and that of sprouting and synapse formation (the intact muscle on the opposite side) made it possible to design experiments to distinguish between the two alternative mechanisms. The current findings suggest a major role for a central mechanism for the induction of contralateral sprouting and synapse formation; the signal appears to arise from the injured nerve cells which communicate it within the spinal cord to intact motoneurones.

Brief communications of some of the observations have been given (Rotshenker, 1977, 1978).

METHODS

Rana pipiens frogs, 5 cm body length, were used.

The preparation. The cutaneous-pectoris muscles of the frog (Fig. 1) are two broad flat muscles situated in the front of the frog's chest. The two distinct muscles are attached to the mid line by a thin layer of connective tissue which also separates them from one another. Muscle fibres originate from the xiphoid process and extend upwards to insert into the skin. Each muscle is supplied by a separate motor nerve whose cell bodies are situated in the spinal cord on the

same side as the muscle they innervate. This was observed in preliminary experiments in which motoneurones to the cutaneous-pectoris muscle were labelled by the retrograde transport of HRP. Also, in an extensive study on the cyto-architecture of the spinal cord of the frog Szekely (1976) found that motoneurones, situated on either the left or right side of the spinal cord give rise to only one axon that emerges through the ventral root on the same side. Motor nerve fibres innervating one muscle do not cross the mid line and therefore do not innervate muscle fibres on the opposite side.

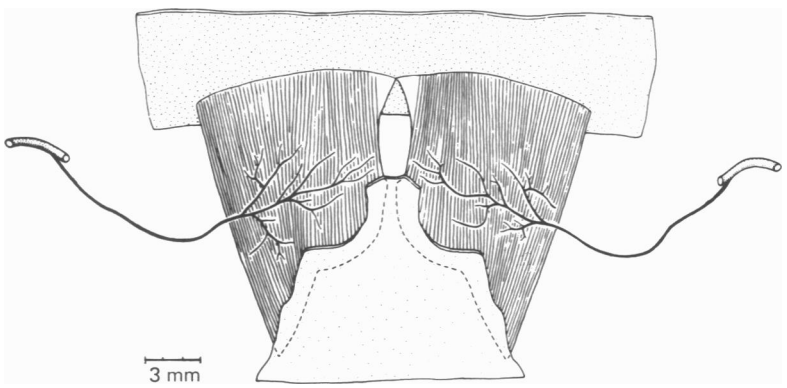

Fig. 1. The cutaneous-pectoris muscles of the frog and their motor nerves as seen from the inside. Muscle fibres originate from the xiphoid (below) and insert into the skin (rostrally). The connective tissue which separates the two muscles is split.

Surgical procedures. Frogs were anaesthetized by placing them in water containing 0·1 % tricaine methanesulphonate (Ayerest). Surgery was performed under a dissecting microscope. The skin was closed with 6–0 silk sutures.

When the left cutaneous-pectoris muscle was denervated, this was done by lesions to either the motor nerve or the brachial nerve from which it is derived. The motor nerve was either crushed by fine forceps or cut by scissors about 1–2 mm from the muscle. The brachial nerve was exposed by a paravertebral incision in the skin and splitting of the muscles overlying it. The nerve was cut about 5 mm from the spinal column.

To remove the left cutaneous-pectoris muscle, the entire muscle was first exposed. Then, the skin was cut above the line to where the muscle fibres are inserted (see Fig. 1). The muscle was lifted at this point and removed by carefully dissecting it from the mid line, from the adjacent muscle on the left and from the muscle underlying it. The two cutaneous-pectoris muscles are separated from one another by a thin layer of connective tissue and this may be cut through without injuring the muscle on the opposite side. However, a few of the muscle fibres that comprise the adjacent muscle on the left side were damaged when the left cutaneous-pectoris muscle was cut away from the xiphoid process. In control experiments where the influence of this muscle damage was examined, muscle fibres in the same region were injured by pinching them with fine forceps or scraping them with a razor blade. Special care was taken not to injure the nerve supply to the muscle.

When the cutaneous-pectoris was 'tenotomized' this was done by cutting the skin all around the line to which muscle fibres are inserted (see Fig. 1). As a result, the muscle with its motor innervation intact was left attached to the xiphoid process. The muscle inserted back to the skin body wall at a lower level than normal in about a week. When the entire left hind limb was denervated all the spinal nerves that innervate it were cut after exposing them by a paravertebral incision of the skin and splitting the overlying muscles. Reinnervation of leg muscles did not occur for 30 weeks at least.

Electrophysiological identification of polyneuronally innervated muscle fibres. For electrophysiological studies both right and left cutaneous-pectoris muscles were removed together with their

common origin (xiphoid process) and insertion (skin) and their motor nerves which are about 1–1·5 cm long (see Fig. 1).

Preparations were bathed in Ringer solution (116 mM-NaCl, 1·8 mM-$CaCl_2$, 2·0 mM-KCl, 1 mM-NaH_2PO_4, dextrose 0·17 %, pH 7·2) which contained curare ($2–4 \times 10^{-6}$ g/ml.). Nerves were stimulated with a suction electrode about 1 cm from the muscle. Evoked end-plate potentials (e.p.p.s) were recorded by impaling muscle fibres with standard micropipettes filled with 3 M-KCl. The micro-electrodes were inserted into the muscle fibres close to their synaptic sites visualized through Nomarksi optics. To identify polyneuronally innervated muscle fibres, brief pulses

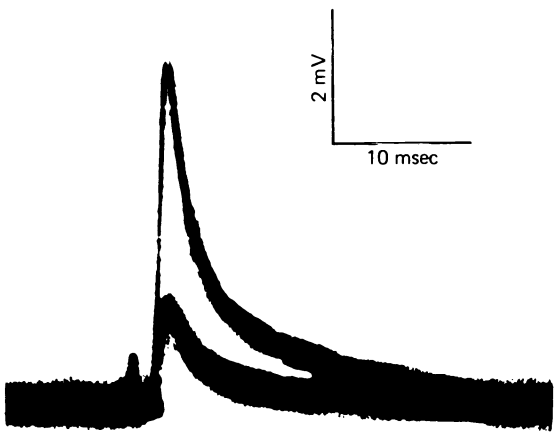

Fig. 2. Polyneuronal innervation. Intracellular recordings of multiple end-plate potentials from a curarized intact muscle fibre. A gradual increase in the intensity of stimulation to the motor nerve elicited the low amplitude e.p.p. first; a further increase in the intensity of stimulation resulted in an abrupt increase in the amplitude of the e.p.p. (see also Methods).

were applied to the nerve at a rate of 1/sec, while gradually increasing the intensity of stimulation. As the increasing stimulus intensity recruited a greater number of axons, there was, in polyneuronally innervated fibres, an abrupt stepwise change in the amplitude of the recorded end-plate potential (e.p.p.); each step was produced by the 'excitation' of a different motor axon (Fig. 2) (see also Redfern, 1970; Brown & Jansen & van Essen, 1976). This method underestimates the incidence of polyneuronal innervation since some thresholds are quite close to another. In addition poorly transmitting synapses will be missed since the preparations were curarized.

Fifty to sixty muscle fibres constituting about 10–15 % of the total muscle fibre population were examined in each muscle. The incidence of polyneuronal innervation for each muscle was defined as the percentage of muscle fibres exhibiting multiple e.p.p.s. The average values were calculated using the individual value from each muscle.

RESULTS

1. The pattern of innervation of single muscle fibres of the adult cutaneous-pectoris muscle of the frog

Axons that comprise the motor nerve to the cutaneous-pectoris muscle of the frog branch after entering the muscle and the branches each innervate individual muscle fibres at a single end-plate area (Letinsky, Fishbeck & McMahan, 1976; Rotshenker & McMahan, 1976). The majority of muscle fibres are innervated by a

single motoneurone and, therefore, when the whole motor nerve to the muscle is stimulated at varying intensities, most muscle fibres exhibit a single end-plate potential (e.p.p.). However, a small proportion, 16% ± 0·5 (S.E. of mean), exhibit multiple e.e.p.s that are evoked by the excitation of axons that arise from different motoneurones (Rotshenker & McMahan, 1976; see also Methods). The pattern of innervation of normal intact muscle can be altered and the incidence of polyneuronal innervation increased. After crushing the motor nerve to the contralateral cutaneous-pectoris muscle, the incidence of muscle fibres exhibiting multiple e.p.p.s increased 1·7-fold over normal to an average value of 26·6 % ± 1·7 (S.E. of mean) (Rotshenker & McMahan, 1976; see also Fig. 4). It was suggested then that the motor nerve to the opposite muscle sprouted and formed additional synaptic connexions with already innervated muscle fibres. Preliminary experiments support this conclusion (Reichert & Rotshenker, 1979). Using the Zinc-iodide-osmium stain (Letinsky *et al.* 1976) it is possible to visualize sprouts arising from axon terminals on one muscle fibre crossing to other muscle fibres whose original innervation can also be seen. Such sprouting is not seen in normal intact cutaneous-pectoris muscles (Letinsky *et al.* 1976).

2. The source of the stimulus for sprouting and synapse formation

Denervation of one cutaneous-pectoris muscle induced sprouting and synapse formation in the opposite intact muscle. The source of the signal for sprouting and synapse formation could be either peripheral or central to the site of the injury. The signal could arise from the denervated muscle or the degenerating axons, as it is commonly suggested to occur in partially denervated muscles (Weiss & Edds, 1946; van Harreveld, 1947; Hoffman, 1951; Edds, 1953; Brown & Ironton, 1977*a*, *b*). Alternatively, the signal for sprouting could arise from the damaged nerve cells central to the site of injury.

The role of injured nerve cells

Axotomy and removal of a muscle. To examine the role injured nerve cells might play in the induction of sprouting and synapse formation, the entire left muscle was removed.

In nine frogs the left cutaneous-pectoris muscle was removed and the right intact muscle examined 8–19 weeks later. The incidence of muscle fibres that had multiple end-plate potentials reached an average value of 34·2 % ± 2·2 % (S.E. of mean) (Fig. 3*A*). Thus the incidence of polyneuronal innervation increased more than twice over normal following neuronal lesion in the absence of the denervated muscle and degenerating axons that innervate it.

The role of injured muscle fibres. During surgery, while removing the left cutaneous-pectoris muscle, a few of the muscle fibres that comprise the adjacent muscle, lateral to the muscles removed, were injured. It could be argued that the damaged muscle fibres induced the formation of the new synapses. The possible role of injured muscle fibres in the induction of sprouting and synapse formation was therefore examined.

In a group of five frogs, the muscle lateral to the left cutaneous-pectoris muscle was injured. The operation was performed under a dissecting microscope and special

care was taken to injure muscle fibres only, leaving the nerve fibres that innervate them intact. It was difficult to quantify the amount of muscle fibres damaged during the removal of the muscle. Therefore, the amount of damage performed in these experiments was based on a subjective estimate to mimic the original damage. Both left and right muscles were examined from 8 to 14 weeks after the operation. The incidence of polyneuronal innervation did not differ from normal in either muscle: 15·0% ± 2·3 (S.E. of mean) and 16·0% ± 1·6 (S.E. of mean) in left and right muscles, respectively (Fig. 3*B*). Therefore, mild adjacent muscle damage is not sufficient to induce sprouting and synapse formation.

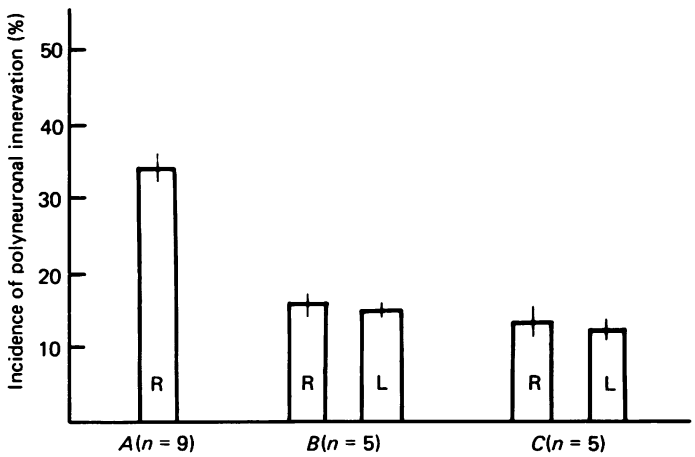

Fig. 3. The effect of removal of the contralateral muscle, of muscle injury, and of immobility, on sprouting and synapse formation. The incidence of polyneuronal innervation in right (R) and left (L) cutaneous-pectoris muscles. *A*, 8–19 weeks after removing the left muscle. *B*, 8–14 weeks after damaging the adjacent muscle to the left cutaneous-pectoris muscle *C*, 4–24 weeks after tenotomizing the left cutaneous-pectoris muscle. *n* is the number of muscles tested in each group. In each muscle fifty to sixty muscle fibres were examined. Bars indicate ± 1·0 S.E. of mean.

The role of immobility. The removal or denervation of one muscle might result in a work overload on the opposite muscle. It has been suggested that such overloading may induce motoneurones to elaborate their axonal endings (Tuffery, 1971). It was of interest, therefore, to examine the effect of immobilization of one muscle on the pattern of innervation in the opposite muscle. In a group of five frogs the left cutaneous-pectoris muscle was 'tenotomized' by cutting the region of skin to which muscle fibres are attached (Fig. 1). As a result, the muscle and its nerve supply were left intact but no tension could develop in the muscle for about 7 days, until it inserted in the skin at a lower level than normal. Both left and right cutaneous-pectoris muscles were examined from 4 to 24 weeks after the operation. The incidence of polyneuronal innervation did not increase over normal in either one of the two muscles, but rather showed a tendency to decrease: 13·5% ± 1·8 (S.E. of mean) in right muscles and 12·5% ± 1·5 (S.E. of mean) in left muscles (Fig. 3*C*). This amount of immobility did not seem therefore to be a sufficient stimulus for inducing contralateral sprouting and synapse formation.

3. The route by which the signal for sprouting is communicated to intact motoneurones

The above results suggest that the damaged nerve cells central to the site of injury are a most likely source of the signal for sprouting and synapse formation. The injured nerve cells could communicate the signal to intact motoneurones either through the spinal cord or systemically via the circulation or by local diffusion of the stimulating material from one cutaneous-pectoris to another. If the signal is communicated in the spinal cord, then sprouting and synapse formation could be

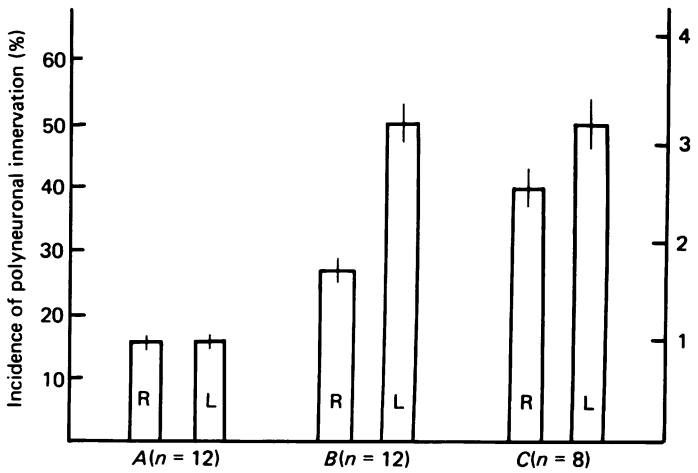

Fig. 4. Nerve transection is more effective than nerve crush in inducing contralateral sprouting and synapse formation. The incidence of polyneuronal innervation in right (R) and left (L) cutaneous-pectoris muscles. *A*, normal; *B*, 8–56 weeks after crushing the motor nerve to the left cutaneous-pectoris muscle. *C*, 8–32 weeks after transecting the motor nerve to the left cutaneous-pectoris muscle. The incidence of polyneuronal innervation is given in the scale on the left and the increase in the incidence of polyneuronal innervation (as multiples of the normal) is given in the scale on the right.

subject to modulation in the same way as are post axotomy changes that occur in somata of injured nerve cells. Chromatolysis, increased synthesis and turnover of nucleotides and proteins and loss of afferent synaptic input are some of the changes that occur in cell bodies of axotomized motoneurones (e.g. Lieberman, 1971, 1974). All these effects are more pronounced if the nerve is transected rather than crushed. Also, the latency with which these changes occur is dramatically decreased if the site of the injury is placed close to the cell bodies rather than further away from them (Watson, 1968, 1974; Kuno & Llinas, 1970*a*, *b*; Lieberman, 1971, 1974; Mendell, Munson & Scott, 1974).

(a) The type of neuronal lesion and the degree of sprouting and synapse formation

Intact muscle. After crushing the motor nerve to the left cutaneous-pectoris muscle the incidence of polyneuronal innervation in the right intact muscle increased 1·7-fold over normal to an average value of 26·6% ± 1·7 (S.E. of mean) (Rotshenker & McMahan, 1976) (see also Fig. 4*B*). A more striking increase in polyneuronal

innervation resulted from a complete transection of the left nerve at the same site. The proportion of muscle fibres exhibiting multiple e.p.p.s increased 2·5-fold over normal and the incidence reached an average value of 39·7 % ± 3·2 (S.E. of mean) (Fig. 4*C*). The time course of the development of the new synapses and their persistence were not influenced by the type of the lesion to the opposite nerve. New synapses were first observed 4–8 weeks after the operation and they persisted for the duration of tests which was up to 56 weeks after crushing the nerve and up to 32 weeks after transecting it. Therefore, the degree of sprouting and synapse formation in one muscle is dependent upon the type of the lesion to the motor nerve to the opposite muscle.

Reinnervated muscle. Crushing the motor nerve to the cutaneous-pectoris muscle at its entry to the muscle is followed by prompt regeneration (as soon as the fourth day after denervation), while a complete transection of the nerve at the same site delayed reinnervation by about 2–3 weeks. The proportion of muscle fibres on the same side that became polyneuronally innervated increased almost 3-fold over normal after crushing the nerve and the incidence reached an average value of 50·1 % ± 3·3 (S.E. of mean) (Rotshenker & McMahan, 1976; see also Fig. 4*B*). Following a complete transection of the nerve there was eventually a similar increase in the incidence of polyneuronal innervation on the same side to an average value of 49·7 % ± 3·8 (S.E. of mean (Fig. 4*C*). Thus, the type of the lesion to the motor nerve did not affect the degree of polyneuronal innervation in the reinnervated muscle, though it delayed reinnervation substantially.

(*b*) *The site of axotomy and the timing of sprouting and synapse formation*

Intact muscle. New synapses in intact right cutaneous-pectoris muscles were detected between 4 and 8 weeks after crushing the nerve to the opposite muscle at its entry into the muscle. By 8 weeks synapse formation reached its maximum value. A similar time course was observed after transecting the nerve at the same site (Fig. 5). However, the latent period between axotomy of the motor nerve to one muscle and the detection of new synapses in the opposite muscle was dramatically shortened when the site of axotomy was placed close to the spinal cord. New synapses were observed as early as nine days after transecting the brachial nerve that contains the motor nerve to the cutaneous-pectoris about 5 mm from the spinal column (Fig. 5). However, the extent of polyneuronal innervation was not influenced by the change in the site of injury. It reached an average value of 39·7 % ± 3·2 (S.E. of mean) after transecting the motor nerve close to the muscle and an average value of 41·4 % ± 1·8 (S,E. of mean) after transecting the brachial nerve (Fig. 5).

Denervated muscle. Reinnervation of the left (ipsilateral) cutaneous-pectoris muscle began 2–3 weeks after cutting the motor nerve just before it entered the muscle. No reinnervation of the denervated muscle was observed following the transection of the brachial nerve for as long as it was tested (10 weeks).

(*c*) *The role of denervated muscle fibres and degenerating axons*

In a group of five frogs the nerve to the left sartorius muscle was cut, and in another group of four frogs the entire innervation to the left hind limb was transected close to the spinal cord. Cutaneous-pectoris muscles were examined 8–30 weeks after the

operation. No alterations were found in the pattern of innervation of either left or right muscles. The incidence of polyneuronal innervation observed in right and left muscles was respectively 15·6% ± 0·8 (S.E. of mean) and 15·6% ± 1·6 (S.E. of mean) after denervating the sartorius muscle and 14·3% ± 0·8 (S.E. of mean) and 15·1% ± 0·8 (S.E. of mean) after transecting the entire innervation to the left hind limb. In the latter case no reinnervation of the left hind limbs was observed for as long as it was tested. Thus, the presence of either a small or large mass of denervated muscles and

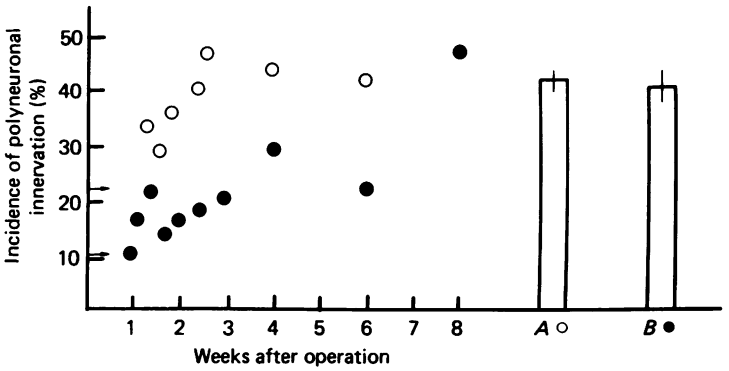

Fig. 5. Lesions near the spinal cord produce earlier contralateral sprouting and synapse formation. The time course of the development of polyneuronal innervation in intact right cutaneous-pectoris muscles as a function of time after transecting the motor nerve to the left cutaneous-pectoris muscle: after transecting the brachial nerve through which the nerve to the muscle passes (○ and *A*) and at its entry to the muscle (● and *B*). Arrows indicate the range within which 95% of the normal values are found (average ± 2 S.D.). The columns present the average plateau values.

degenerating axons did not induce supernumerary innervation. Therefore, sprouting and synapse formation are unlikely to be induced systemically. Furthermore, the intact motoneurones to the cutaneous-pectoris muscle do not respond with new synapse formation if they and the damaged neurones are situated at a distance of several segments from each other in the spinal cord.

DISCUSSION

The present results suggest that the signal for the sprouting of the intact motoneurones to one cutaneous-pectoris muscle arises from the injured nerve cells of the motor nerve to the opposite muscle central to the site of axotomy. The signal appears then to be communicated transneuronally across the spinal cord from the damaged neurones to the responding intact motor nerve cells.

Several lines of evidence support this conclusion. First, sprouting and synapse formation occurred in the absence of denervated muscle fibres and degenerating axons, thus leaving the damaged neurones as a most likely source for the signal for sprouting. Secondly, placing the site of axotomy away from the denervated muscle rather than close to it, shortened the delay with which new synapses appeared in the intact muscle. The timing of the response was thus modulated in the opposite direction from what is expected if the source of the signal for sprouting and synapse formation

was the denervated tissue. The onset of many of the denervation changes that follow axotomy is slowed when a longer nerve stump is left attached to the denervated muscle; degeneration of nerve fibres and cessation of transmitter release (Birks, Katz & Miledi, 1960; Miledi & Slater, 1970), spread of acetylcholine sensitivity (Luco & Eyzaguirre, 1955; Emmelin & Malm, 1965), appearance of tetrodotoxin-resistant action potentials (Harris & Thesleff, 1972), decrease in resting potential (Alburquerque, Schun & Kauf, 1971) and decrease in end-plate cholinesterase (Davey & Yonkin, 1978). The decreased delay with which sprouting and synapse formation followed the placement of axotomy close to the spinal cord suggests therefore the involvement of the cell bodies and central processes of the damaged nerve cells in the spinal cord. The increased magnitude of sprouting and synapse formation that followed the more severe injury (transection versus crush) further supports this conclusion. Similar observations were made on the dependence of the magnitude and time of onset of post axotomy changes in somata of injured nerve cells on the type and the site of axotomy; chromatolysis (*e.g.* Lieberman, 1971, 1974), increased synthesis and turnover of nucleotides and proteins (Watson, 1968, 1974), changes in electrophysiological properties and loss of afferent synaptic input (Kuno & Llinas, 1970*a*, *b*; Mendell *et al.* 1974). Finally, the presence of a large mass of denervated muscles and degenerating nerves that followed the complete denervation of a hind limb did not cause any alterations in the pattern of innervation of the intact cutaneous-pectoris muscles, thus suggesting that the formation of the new synapses is not induced systemically and furthermore that motoneurones do not respond to the injury of neurones situated in distant segments of the spinal cord.

Injury of nerve cells can thus promote a growth response which apparently acts transneuronally in the central nervous system. It differs in that respect from what is suggested to occur in partially denervated tissues. There, local interactions, in the periphery, between the sprouting axons and the denervated tissue (denervated target cell and/or degenerating axons) are believed to be the primary cause for sprouting and synapse formation. It was suggested that in muscles, the stimulus for sprouting may be products arising from degenerating axons (Hoffman, 1950, 1951; see also Edds, 1953) or from denervated muscle fibres (van Harreveld, 1947), possibly as a consequence of the state of inactivity that follows the axotomy (Brown & Ironton, 1977*a*, *b*). In the salamander, sprouting of sensory nerve fibres was proposed to result from the lack of a neurotrophic substance that normally neutralizes a growth promoting action of a material liberated by the target cells (Aguilar, Bisby, Cooper & Diamond, 1973; Diamond, Cooper, Turner & MacIntyre, 1976). It is impossible to tell at present whether interactions between nerve and muscle such as those described above also participate in the formation of the new synapses in the intact cutaneous-pectoris muscle. However, if they were to play a role then they would be secondary to and activated by the primary stimulus that arises from the injured nerve cells on the opposite side.

The nature of the signal that arises from the injured nerve cells and induces sprouting and synapse formation in the intact cutaneous-pectoris muscle remains unclear. There are, however, indications for the possible involvement of trophic interactions between nerve and muscle. The dependence of the timing of the response upon the length of axon that remains attached to the damaged neurone suggests that the

critical event that follows axotomy may be the reduction in concentration of some trophic substance which is normally produced in the peripheral field of the damaged neurone and transported retrogradely to the cell soma. A similar mechanism has been suggested to operate in another transneuronal event – the maintenance of synapses on sympathetic (Nja & Purves, 1978) and motor nerve cells (Sumner & Sutherland, 1973; Cull, 1973, 1974). Another possibility is that sprouting and synapse formation result from the altered pattern of activity of the intact motoneurones (Brown & Ironton, 1977*a*, *b;* however, Benoit & Changeux, 1978) that follows the change in activity of the injured nerve cells that may synapse directly or indirectly with the responding motoneurones on the opposite side. Further experiments are needed to resolve this question of the nature of the stimulus for sprouting and synapse formation.

The present results suggest then that the peripheral field of innervation of the motor nerve cells that innervate one cutaneous-pectoris muscle is influenced by the injured neurones that innervate the opposite muscle. The precise mechanism by which this influence is exerted is yet unknown. This finding raises, however, the interesting possibility that the pattern of innervation of a tissue may be dependent not only on the interactions between the innervating nerve cells and their target cells but also on the integrity of other neurones with which the innervating nerve cells can communicate in the central nervous system.

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