J. exp. Biol. 132, 231–247 (1987)
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STRENGTHENING OF SYNAPTIC INPUTS AFTER ELIMINATION OF A SINGLE NEURONE INNERVATING THE SAME TARGET

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SUMMARY

The problem of 'competition' between neurones innervating the same target can be studied in simple neural systems such as the central nervous system of the leech and the lobster neuromuscular junction. Intracellular injection of pronase to kill selectively a single neurone shows that, in the leech, removal of one neurone is a sufficient signal to produce compensatory changes. After removal of a given neurone, only neurones of the same function respond to innervate the 'vacant territory'. This was shown both for a motor neurone (annulus erector) and sensory neurones (T or N). Thus the response is very specific.

The lobster neuromuscular junction, with its multiple excitatory and inhibitory innervation, has advantages for the study of changes in synaptic efficacy of the remaining neurones after removal of a defined neurone releasing the same or a different transmitter. Killing the inhibitory neurone produced prolongation of the excitatory synaptic current because of a prolonged channel open time. When an excitatory axon is killed the remaining excitatory axon releases more transmitter. Over a period of 10 days, there is first a strengthening of existing synapses, then the appearance of new release sites and sprouting. Only those terminals of a neurone that innervate a territory with reduced innervation become stronger, while other terminals of the same axon remain normal. Cutting of axons produces different responses from those seen after killing single neurones.

INTRODUCTION

The concept of 'competition' between neurones, especially during development, implies that neurones compete for limited quantities of specific factors which influence growth, the development of contacts with their targets and their synaptic strength (Purves, 1977, 1986; Liestol, Maehlen & Nja, 1986; Hubel & Wiesel, 1965; Easter, Purves, Rakic & Spitzer, 1985). Competition appears to play a decisive role in the development and organization of the mammalian and vertebrate nervous system. However, because of the constancy found in the invertebrate CNS, it has been suggested that competition may have a lesser role in the reshaping of this nervous system (Easter *et al.* 1985). The latter interpretation is challenged by Murphey

Key words: synapse, competition, synaptic efficacy, plasticity.

(1986) who argues that competitive interactions do occur at least in some invertebrates (e.g. in the leech and arthropods). He analysed different aspects of competitive interactions and demonstrated that such processes are involved in determining the number of neurones during development, activity-dependent synaptogenesis and synaptic organization during maturation (see also Murphey & Lemere, 1984; Shepherd & Murphey, 1986).

In this chapter, I will discuss some examples from relatively simple invertebrate nervous systems which enable the study of mechanisms controlling plastic changes (Jansen, Muller & Nicholls, 1974; Wallace, Adal & Nicholls, 1977) that may involve competitive interactions. Some interactions which occur between neurones in the *adult* nervous system of leeches and lobsters will be considered in this paper. It is often stated that competition between neurones occurs mainly during a 'critical period' of development (Hubel, Wiesel & LeVay, 1977), and that neurones and their connections become more rigid with age. Nevertheless, neurones do die or are damaged in the adult nervous system and compensatory mechanisms for such losses must exist in vertebrates as well as in invertebrates. Since neurones also die during development, it is possible that some of these compensatory mechanisms found in the adult also operate during development. The advantage of the invertebrate nervous system is that the simpler system enables a more quantitative and biophysical analysis of some of the changes which occur due to damage, use or disuse. Some of these mechanisms may also exist in the more complicated nervous systems of vertebrates.

REMOVAL OF SINGLE NEURONES

In vertebrates, severing a nerve trunk produces degeneration of the peripheral stump. Thus, the cutting of nerves (or ablations) is a major technique used to produce changes in the nervous system. Processes which occur during regeneration are considered in many cases to recapitulate those which occur during development (Purves, 1977). The technique of cutting nerves is less useful in invertebrates, as in many cases it does not produce degeneration of the peripheral stump (Hoy, 1969; Hoy, Bittner & Kennedy, 1967; but see Bittner & Johnson, 1974). Since the processes of a neurone may extend over several ganglia (Nicholls & Baylor, 1968; Baylor & Nicholls, 1969) and synaptic inputs occur on neurite processes, the flow of synaptic potentials and action potentials to other neurones is not mediated through the soma. Therefore, neural activity continues even if the neurite is disconnected from its soma. Nevertheless, the central nervous systems of some invertebrates are very highly stereotypic, the locations and shapes of neurones are more or less constant, and synaptic inputs to some neurones are well characterized. In many cases, neurones receive several synaptic inputs, some of which are always 'strong' (criterion: amplitude recorded in the soma) while others are always weaker. This relatively precise and constant organization is especially convenient for the study of mechanisms controlling the shape and field of innervation of neurones, as well as those that determine the synaptic strength of a given input. For example, if a neurone

is innervated by two synaptic inputs, one strong and the second weak, will the weak synapse become stronger upon removal of the strong input? To be able to answer such a question, a technique is required to remove a neurone and all its processes completely and to test for changes in the remaining neurone innervating the same target.

It is clear that surgical removal of a neurone with all its arborizations without inflicting damage to other neural arborizations is technically impossible. Destroying a soma with a fine laser beam does not help because the decapitated neurite often continues to function for many months. One way to overcome these difficulties was suggested by Parnas & Bowling (1977) and Bowling, Nicholls & Parnas (1978). A small amount of the proteolytic enzyme, pronase, is injected intracellularly, the enzyme diffuses into the arborizations of the injected neurone and lyses it without producing overt damage to remaining adjacent neurones. Extensive controls were performed to show that pronase does not lyse cells when injected in small quantities into the extracellular space, and that leakage of pronase from the disintegrating neurone does not affect the terminals of adjacent cells. By using double injections, first of horseradish peroxidase (HRP) and a few hours later of pronase, we could visualize the disintegration of the very fine terminals of the injected neurone (Bowling *et al.* 1978).

This technique enabled us to examine some basic mechanisms involved in the compensation for such a microlesion. For example, if a neurone is removed, will other neurones expand into the vacant territory and take over the missing function? If other neurones do respond, will they be the nearest neurones in the denervated region regardless of their function, or will only neurones of the proper function take over?

In the leech nervous system both motor and sensory neurones have precise fields of innervation in different ganglia (Baylor & Nicholls, 1969). Thus, this system is ideal for the study of the above questions.

REMOVAL OF THE ANNULUS ERECTOR MOTOR NEURONE

The first cell to be deleted was the annulus erector motor neurone (Parnas & Bowling, 1977; Bowling *et al.* 1978). There is a pair of such neurones in the posterior part of each ganglion and each cell innervates the annulus erector muscles on the contralateral side. The field of innervation of each neurone extends from the dorsal midline to the ventral midline over a length of nine annuli; five in its own segment and two in each of the anterior and posterior adjacent segments (Fig. 1A; Stuart, 1970). Thus, fields of innervation of annulus erector neurones in adjacent ganglia overlap. As can be seen from Fig. 1A, removal of annulus erector cells on one side, in two successive ganglia, would be expected to produce the loss of the erector reflex in six annuli. Fig. 1B shows that this was the result after such an injection. When injected animals were kept for a few months, the annulus erector reflex reappeared. These animals were tested to find which neurones reinnervated the vacant territory. We were surprised to find that the remaining two annulus erector cells in the injected

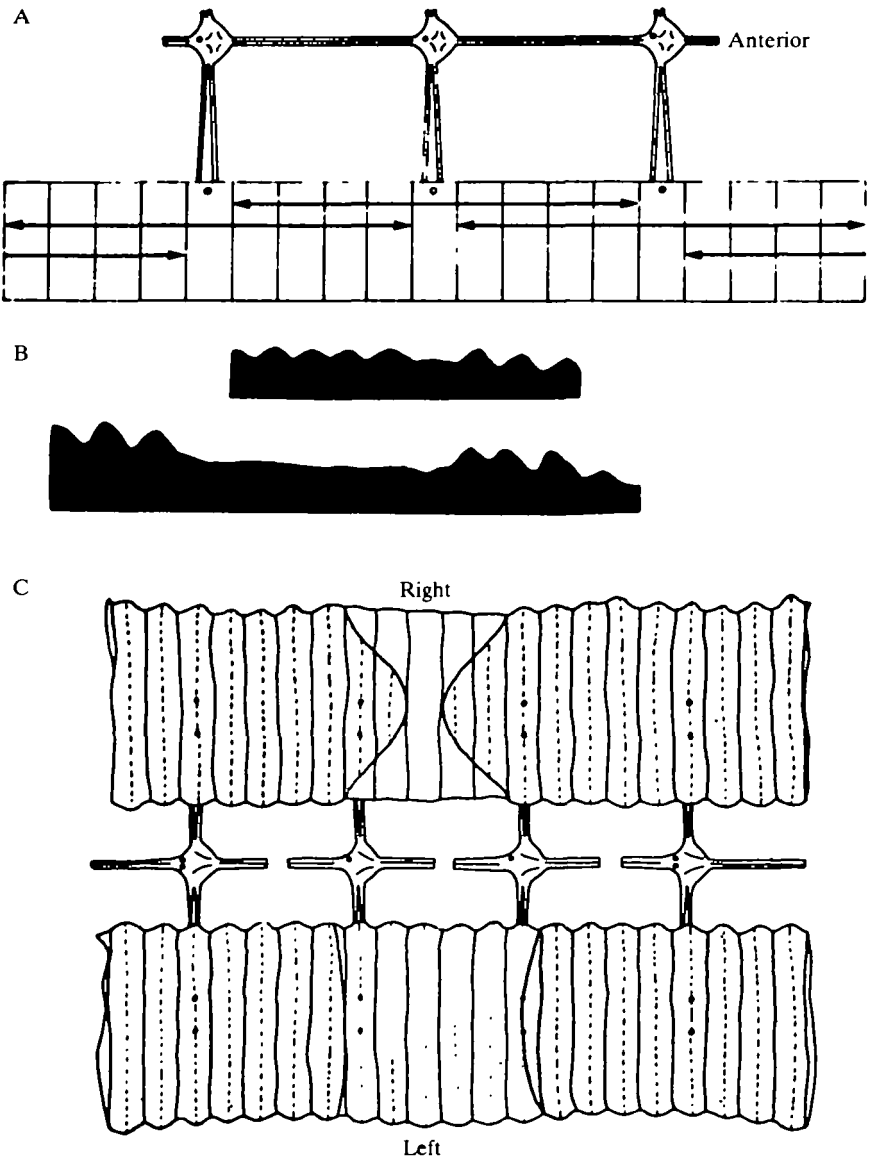


Fig. 1. (A) Schematic representation of fields of innervation of annulus erector cells. Each neurone innervates the five annuli of its own segment and two annuli in the adjacent anterior and posterior ganglia. (B) Enlarged section of body wall of the leech. The photograph was taken after the animal had been stimulated to evoke the annulus erector reflex. Top: after removal of one cell, the reflex was lost, as expected, in only one annulus. Bottom: after removal of two cells in successive ganglia, the reflex was lost in six annuli. (C) Recovery of the reflex occurred by expansion of fields of innervation to the denervated region of annulus erector cells in the anterior and posterior ganglia. (From Bowling, Nicholls & Parnas, 1978.)

ganglia did not expand their fields across the ventral and dorsal midlines. In addition, the L motor neurones which innervate the longitudinal muscles did not invade into the annulus erector muscles, even though they run close to each other. Instead, we found that the fields of innervation of the anterior and posterior annulus erector cells, on the same side in the adjacent ganglia, expanded their fields to take over the vacant territory (Fig. 1C).

This type of a response requires a very specific signal, which not only distinguishes between the annulus erector cells and other neurones, but also recognizes the left and right sides.

REMOVAL OF SENSORY NEURONES

The fields of innervation of the sensory neurones T, N and P (Baylor & Nicholls, 1969) are so precise that these cells can also be used to test for the responses after elimination of neurones of a given modality. It should be emphasized that in controls, the T, N and P cells of one side never cross the dorsal or ventral midlines. After removal of the three T cells on one side, the dorsal T cell from the other side crossed the dorsal midline to innervate the denervated skin (Blackshaw, Nicholls & Parnas, 1982). It is interesting that the response was again specific, N or P cells did not expand their fields of innervation. When the two N cells on one side were removed, the remaining contralateral N cells expanded their fields of innervation across the dorsal or ventral midlines. Removal of one N cell was not sufficient to allow for the expansion of fields of innervation. In other words, the remaining N cell on one side was sufficient to prevent expansion of the fields of innervation of the N cells on the other side. Removal of the two N cells also did not affect the fields of innervation of P or T cells. We therefore conclude that upon removal of a specific neurone, only neurones of the same modality respond to expand their field of innervation to compensate for the missing function. In contrast to the annulus erector cells, the sensory neurones compensated for the missing function by crossing the dorsal and ventral midlines.

The signal for such a specific response is not known. However, it cannot be simply the result of reduced electrical activity as only cells of the proper function expanded their fields of innervation. Other neurones which were nearer to the denervated region did not respond, and did not expand their fields of innervation.

CHANGES IN SYNAPTIC EFFICACY

In the central nervous systems of invertebrates, synaptic inputs are located on the neurite at some distance from the soma. Recordings of synaptic potentials or currents are made in the soma, as the neurites are too small and cannot be isolated from other neurite terminals of other neurones in the neuropile. This organization is less favourable for biophysical analysis of changes in synaptic inputs. Nevertheless, Shepherd & Murphey (1986) succeeded in demonstrating changes in synaptic

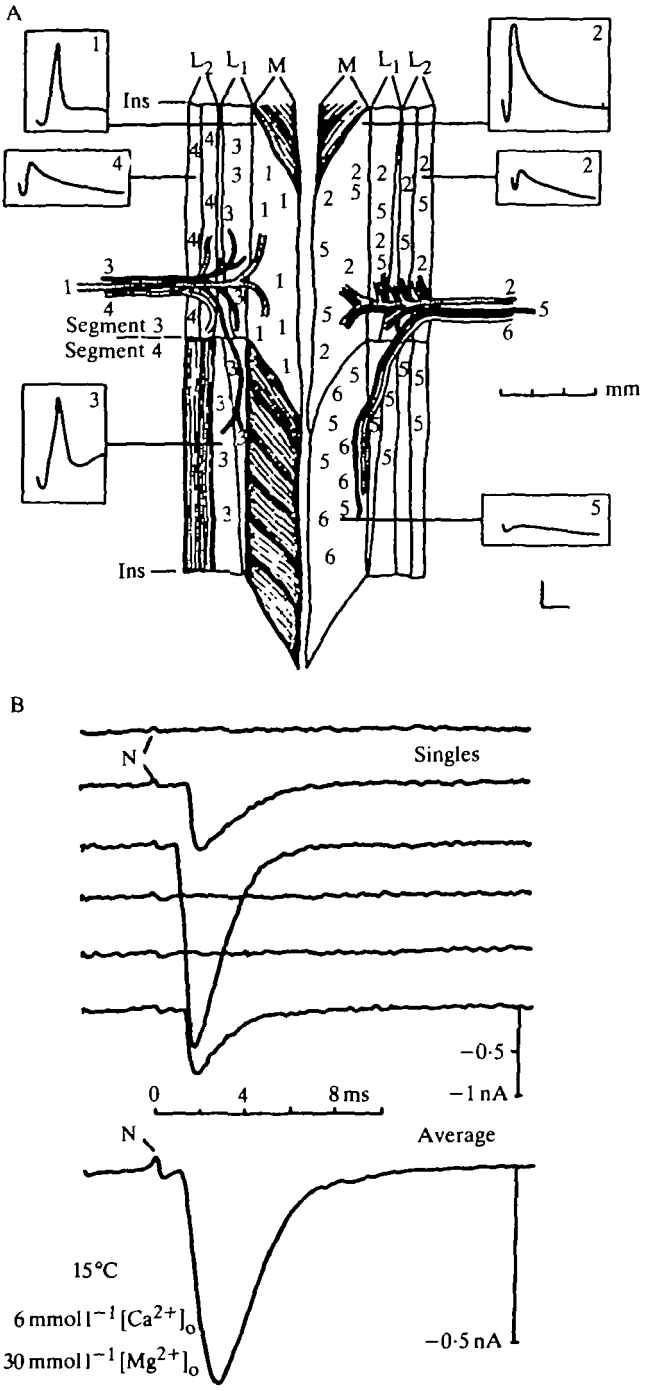
efficacy in an identified neurone in the cricket when neighbouring afferent synapses were removed.

Neuromuscular systems are convenient for recording synaptic potentials or synaptic currents, and in several cases the neuromuscular systems of frogs and mammals have been used to study a variety of interactions between the motor axons and the muscle (Brown, Jansen & Van Essen, 1976; Kuffler, Thompson & Jansen, 1977; Rotshenker, 1979, 1982; Rotshenker & Tal, 1985; Schuetze & Vicini, 1984; Brown, Holland & Hopkins, 1981; Pecot-Dechavassin, 1986). The neuromuscular system of vertebrates, although being convenient for the study of synaptic transmission, is limited as a model for the study of processes that may occur in the central nervous system. In the central nervous system, neurones receive many inputs releasing the same or different transmitters. In vertebrates, most muscle fibres receive a single innervation, and only one transmitter substance is involved. Therefore, questions as to interactions between neurones innervating the same target cannot easily be addressed using the vertebrate neuromuscular junction.

In crustaceans, muscle fibres are innervated by more than one excitatory axon and also by inhibitory axons (Atwood, 1977). The excitatory transmitter is glutamate (Kawage, Onodera & Takeuchi, 1982), the inhibitory transmitter is gamma-aminobutyric acid (GABA) (Otsuka, Iversen, Hall & Kravitz, 1966). In addition to the polyneuronal innervation, the crustacean neuromuscular system exhibits multi-terminal innervation (Atwood, 1977), i.e. the axon passing along the muscle has many release sites. Furthermore, the whole muscle is innervated by one or a few excitatory axons, and one or two inhibitory axons. The crustacean neuromuscular system is thus favourable for the study of plastic changes during development (Atwood & Kwan, 1976) or after activity (Atwood & Wojtowicz, 1986).

The deep abdominal extensor muscles (DEAM) are composed of three bundles M, L_1 and L_2 (Parnas & Atwood, 1966). Each bundle is innervated by a specific excitatory axon (ExM, ExL_1 and ExL_2), by a common excitor (Com.Ex.) and a common inhibitor (Com.In.). The field of innervation of each axon is well recognized (Parnas & Atwood, 1966; Fig. 2A). Synaptic potentials can easily be recorded intracellularly and synaptic currents can be recorded from a single or a few release sites (Fig. 2B) using the 'macropatch' technique (Dudel, 1981). The macropatch electrode can be slid along the muscle fibre and the distance between releases sites determined. In the abdominal extensor muscles release sites appear about every 100 μm (Parnas, Dudel, Cohen & Franke, 1984). In addition, the axons

Fig. 2. (A) Scheme of innervation of the deep abdominal extensor muscles (DEAM) in the lobster. On each side of the tail the DEAM are composed of three bundles, the medial, M, and the two lateral bundles, L_1 and L_2. In each segment, the nerve to the DEAM is composed of five axons. A common excitor (Com.Ex.), a common inhibitor (Com.In.) and the specific excitors (ExM, ExL_1 and ExL_2). ExM, ExL_1 and Com.In. each send a posterior branch to innervate the next segment. (From Parnas & Atwood, 1966.) (B) Samples of synaptic currents recorded using the macropatch electrode. Recordings taken from the L_1 muscle in segment II. Top: traces of responses to single stimuli. The nerve response, N, can be seen. Second trace, one quantum response; third trace, two quanta. Bottom: average of 128 traces. (From Parnas, Dudel, Cohen & Franke, 1984.)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are relatively large and can be impaled by microelectrodes for injection (Parnas, Dudel & Grossman, 1982; Parnas *et al.* 1984). Therefore, this system can serve as a useful model to study interactions between axons releasing the same or different transmitters and innervating the same target.

REMOVAL OF THE INHIBITORY NEURONE

The common inhibitor was eliminated by intracellular injection of pronase (Parnas *et al.* 1982). 10–16 days after injection, the excitatory synaptic potentials were longer than normal, showing a slower decay. This change was not seen if the muscles were tested 3–5 days after injection. It can be concluded that the prolongation of the decay time of the EPSP was not produced by a mere increase in membrane resistance (which develops immediately) resulting from the removal of tonic inhibition (Parnas, Rahamimoff & Sarne, 1975). Injection of pronase into one excitatory axon did not produce this change in the synaptic potentials of the remaining excitatory axon.

The amplitude and time course of a synaptic potential depend on the synaptic current as well as on the muscle membrane resistance. The time course of the synaptic current does not depend on membrane resistance. We therefore measured synaptic currents which now showed 2–4 times slower decay (Parnas *et al.* 1982). Such a result could have arisen from asynchronized release of transmitter, from slowing of the removal of glutamate, or from a longer channel open time. Single quanta released spontaneously also showed the same slower time course. Therefore, the possibility of asynchronized release of quanta as the only reason for slower decay is unlikely. The decay of the slowed synaptic current showed a single exponential, making the possibility of slowed removal processes less probable. We are, therefore, left with the possibility that removal of the inhibitory axon resulted in prolongation of the channel open time of the excitatory channel. Recording of single-channel activity from the deep extensor muscles is possible (Franke, Hatt & Dudel, 1986) and preliminary experiments have shown that the channel open time is almost doubled after the removal of the inhibitory axon (C. H. Franke, J. Dudel, I. Cohen & I. Parnas, unpublished observations).

This is the first time that such an interaction between two axons innervating the same target and releasing different transmitters has been demonstrated. Since it took 10–16 days for the change to develop, two mechanisms could be involved. Either existing channels prolonged their open time or new receptors with slower channels could have been inserted into the membrane.

STRENGTHENING OF TRANSMITTER RELEASE

As stated above, each of the bundles of the deep extensor muscles is innervated by two excitatory axons. In the L_1 bundle the common excitor produces a large synaptic potential and the specific excitor ExL_1 a small synaptic potential (Parnas & Atwood 1966). Thus, this system can serve for the study of the effects of removal of a 'strong' axon on the weaker remaining axon terminals. At first, control values of the

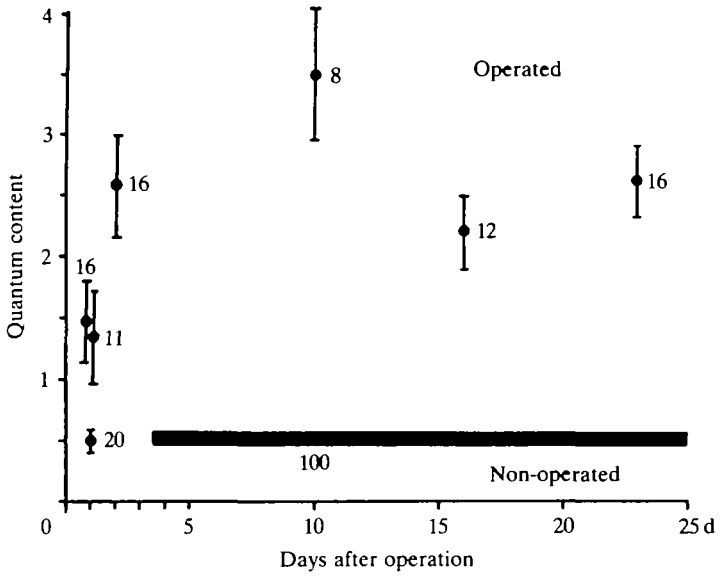

Fig. 3. Strengthening of release by the ExL_1 axon after removal of the common excitor (Com.Ex.). Solid bar shows control levels of release; 100 measurements taken from seven muscles. The average was 0·5 quanta. After removal of the Com.Ex., release increased over a period of about 5 days. The numbers near each point represent the number of synapses from which recordings were taken. The bar indicates the standard error. (From Parnas, Dudel, Cohen & Franke, 1984.)

magnitude of release from single or a few release sites were determined using the same macropatch electrode in all experiments (Parnas *et al.* 1984). Recordings were made in a limited region of the L_1 muscle on both sides, and the quantal content was determined for at least 128 nerve impulses. We found that the control quantal content varied between 0·2 and 1, and the responses in the two muscles were about the same. This is fortunate, because one muscle can then serve as a control for the second injected side.

After removal of the common excitor on one side, transmitter release of ExL_1 was measured both on the injected and the control sides. Fig. 3 shows a clear increase in release that developed with time. Release doubled 24–48 h after injection, and was 5–10 times higher than the control after 10 days. Strengthening of existing release sites was the first effect. During the first few days following injection, the distance between release sites was still about 100 μm. With time, the distance between release site was reduced to 50 μm, and hence it became much easier to find a release site. Staining such preparations with methylene blue showed extensive sprouting of the remaining ExL_1 axon terminals. These were only limited to the original territory of innervation of ExL_1, and did not cross to other segments or sub-bundles. Many more axon terminal profiles could be seen in electron micrographs, and in a few cases structures resembling growth cones were identified. It is not clear whether those additional release sites, found before sprouting, were indeed new or whether they were silent sites activated by the removal of the excitatory axon. Electron

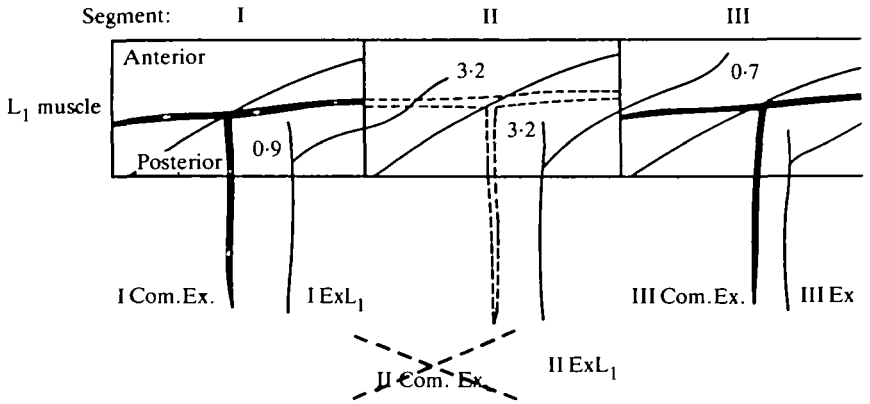

Fig. 4. A scheme of innervation of the L_1 bundles by the common excitor (Com.Ex.) and ExL_1 in three successive segments. In each segment, the muscle is divided by a cleft into an anterior and a posterior sub-bundle. The Com.Ex. in each segment innervates both sub-bundles, and it does not innervate fibres in adjacent segments. The ExL_1 in each segment innervates only the posterior sub-bundle, but also sends a branch to innervate the anterior sub-bundle of the following segment. In segment II, the Com.Ex. is shown by a broken line as it was removed by pronase injection. Note that, now, terminals of ExL_1 of segment II innervate fibres in segment II without Com.Ex. innervation, while the posterior branch innervates fibres in segment III with Com.Ex. innervation. The ExL_1 axon from segment I innervates normal fibres in segment I and partially denervated fibres in segment II. The numbers show the average quantal content established in 13 preparations. Note that in the posterior sub-bundle of segment I and the anterior sub-bundle of segment III, the average quantal contents were 0·9 and 0·7, respectively. In segment II the average was 3·2. (From Dudel & Parnas, 1987.)

micrographs have not as yet revealed more release sites nor could shorter distances between regions which can be classified as typical synaptic release zones be found. However, serial sections and three-dimensional reconstruction are required to observe such changes, and this was not done.

The signal for such synaptic strengthening is not clear. It may be a central signal as demonstrated by Rotshenker (1979, 1982) for the cutaneous pectoris muscle of the frog. In that study, cutting the nerve on one side produced sprouting in the contralateral muscle. The signal could be peripheral, where the closely apposed terminals of the two axons may compete for a limited amount of some factor or factors. Alternatively, the terminals may produce a factor which reciprocally reduces release. Removal of one axon diminishes this regulatory factor and, hence, release by the remaining axon would increase.

It is possible to distinguish whether the signal is central or peripheral because of the pattern of innervation of the L_1 bundles by ExL_1 axons in successive segments (Parnas & Atwood, 1966). Fig. 4 shows a scheme of the innervation pattern of the L_1 bundle in three successive segments. For clarity only the two excitatory axons are shown. In each segment, there is a cleft dividing the L_1 bundle into anterior and posterior sub-bundles. In each segment, the Com.Ex. axon innervates the two sub-bundles. The ExL_1 of a given segment innervates only the posterior sub-bundle of that segment, but also sends a branch to innervate the anterior sub-bundle of the

immediate posterior segment. Thus, each muscle fibre in a segment is innervated by two axons. Those fibres in the posterior sub-bundles are innervated by axons originating only from the corresponding ganglion, whereas those in the anterior sub-bundle receive innervation from two ganglia. Fig. 4 also shows that removal of the Com.Ex. in segment II produces different conditions for the ExL_1 axon terminals of segments I and II innervating the different sub-bundles in segments I, II and III. The terminals of ExL_1 originating in segment I innervate the posterior sub-bundle of segment I muscle fibres, which are also innervated by a common excitor. Terminals of the same axon, innervating the anterior sub-bundle of segment II, innervate fibres which now lack Com.Ex. innervation. Similarly, terminals of ExL_1 originating in segment II, innervate the posterior sub-bundle of segment II, which now lacks Com.Ex. innervation, while the posterior branch innervates fibres with Com.Ex. innervation in segment III.

If the signal for strengthening the release of transmitter is central, i.e. originating in the ganglion of segment II, it is reasonable to expect that the terminals of ExL_1 originating in segment II would become stronger both in the posterior sub-bundle of segment II and in the anterior sub-bundle of segment III. The terminals of ExL_1 from segment I would be expected to remain normal. However, if the signal for strengthening is peripheral, i.e. resulting from partial denervation of the muscle fibres in segment II, it would be expected that the terminals of ExL_1, originating in ganglia I and II and innervating segment II (the two sub-bundles) would be stronger, whereas other terminals of these same two axons innervating segments I and III, with full Com.Ex. innervation, would remain normal.

The results (Dudel & Parnas, 1987) were very clear. Only those ExL_1 axon terminals that innervated the two sub-bundles in segment II became stronger (Fig. 5), whereas the terminals in segments I and III remained normal, releasing on average 0·5–1 quanta per impulse. Therefore, not only is the signal for strengthening synaptic release peripheral, but it must be localized as well. The posterior branch of ExL_1 originating in segment I, on its way to segment II, also innervates fibres in the posterior sub-bundle of segment I. We have recorded synaptic currents on both sides of the border between segments I and II. All responses in segment I were normal, whereas high release was observed upon a small shift of the electrode, by about 100 μm, to segment II. We therefore conclude that the signal for the strengthening of synaptic release is produced locally and does not diffuse over great distances.

What is producing the signal? Is it the actual removal of the axon terminals, e.g. partially denervating the postsynaptic target cell, or is it the reduced electrical activity which results from partial denervation? The role of activity in establishing synaptic contacts has been shown by Stryker (1981) and Boss & Schmidt (1984). This question can also be answered directly in the L_1 neuromuscular system since after cutting the nerve the peripheral stump does not degenerate but is electrically silent (at least for action potentials). The nerve innervating the extensor muscles was cut in segment II (Dudel & Parnas, 1987) and the release from the posterior branch of the ExL_1 axon, originating in segment I and innervating segment II, was

measured. The release from these terminals remained normal for a period of at least 10 days after the cut. It seems that the presence of the Com.Ex. terminals in segment II, which were weaker after the cut (Parnas *et al.* 1984) and electrically

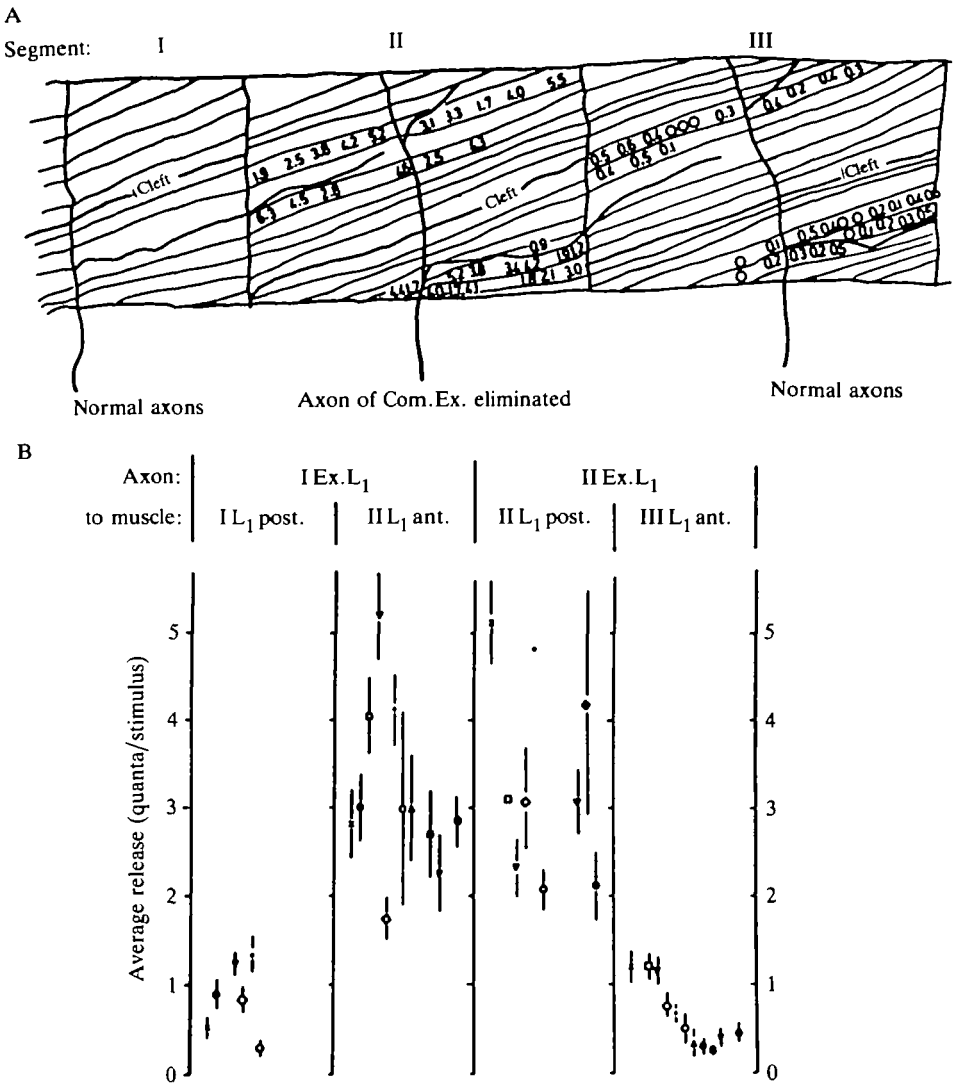

Fig. 5. Quantal content established in the different sub-bundles after removal of the common excitor (Com.Ex.) in segment II. (A) Values of quantal content and sites of recording in one experiment. Note that the values of the quantal content in segment II are high for both the posterior branch from segment I and the ExL_1 from segment II. The values ranged from 0·9 to 6·3. In segment III, where the Com.Ex. was intact, the quantal content of the ExL_1 terminals varied between 0·1 and 0·6. (B) Graphic representation of quantal content established in the different sub-bundles of segments I, II and III from 13 preparations. The average of each point is made up from 2–22 determinations of release rates at different locations. Vertical bars show standard deviation. For each site at least 128 nerve impulses were measured. (From Dudel & Parnas, 1987.)

silent, was sufficient to prevent the strengthening of release from the ExL_1 axon terminals.

The molecular mechanisms involved in the strengthening of the synaptic response are not clear at present. Our results (Dudel & Parnas, 1987) are compatible with the concept that the target organ produces a trophic factor which is limited in amount in order to support all of its inputs. Trophic factors are known to be involved in the establishment of synaptic contacts (Thoenen & Barde, 1980; Nja & Purves, 1978; Csillik, Schwab & Thoenen, 1985). If this is the case, then the inactive and weaker terminals of the cut Com.Ex. were still able to deplete some of these trophic factors. It is also possible that terminals of adjacent axons release factors which suppress release by the other terminals (and therefore, also by themselves). Whatever the mechanisms may be, they must act over very short distances of only a few micrometres.

LONG-TERM EFFECTS OF NERVE CUTS

While performing the experiments with cut nerves described above, we noticed longer-term changes that were not seen when single axons were totally removed by the intracellular injection of pronase. Shortly after cutting the nerve, the release of transmitter from the severed peripheral stumps became weaker (Parnas *et al.* 1984) and release sites could only be found near the main trunk of the nerve, as it enters the L_1 muscle. Therefore, even though the terminals did not degenerate, i.e. they could be identified in electron micrographs, their release properties were altered.

After longer periods (8–67 days) EPSPs were again recorded in the muscle in response to stimulation of the cut nerve in segment II. However, now instead of the two sub-levels of EPSPs normally observed when the Com.Ex. and ExL_1 axons were recruited with increasing stimulus intensity, 4–6 sub-levels of EPSPs were recorded (Fig. 6). This result shows that more than two excitatory axons innervated one fibre in the L_1 muscle. Cross-sections of the cut nerve showed the three axons normally found (Com.Ex., ExL_1, Com.In.), but also revealed new axon profiles. The origins of these new axons are not clear. Two of the axon profiles can still be attributed to the old Com.Ex. and ExL_1, but the others must be branches of axons that do not normally innervate the L_1 muscle (I. Cohen, I. Parnas & J. Dudel, in preparation). In some cases a new nerve growing into the L_1 muscle was observed. The changes seen after cutting the nerve varied among animals. The variation was in the number of new axons innervating the different fibres of a given muscle and in the pattern of nerve sprouting.

The posterior branch of the nerve coming from segment I also changed. This branch normally contains branches of three axons (Com.In., M axon and ExL_1). Stimulation of this branch produces only one EPSP in the anterior sub-bundle of the L_1 muscle in segment II. After the nerve in segment II had been cut, we found that stimulation of the posterior branch of segment I produced up to six levels of EPSPs in segment II. More striking was the growing and sprouting of these new axons that crossed the partition to innervate the posterior sub-bundle in segment II, which also

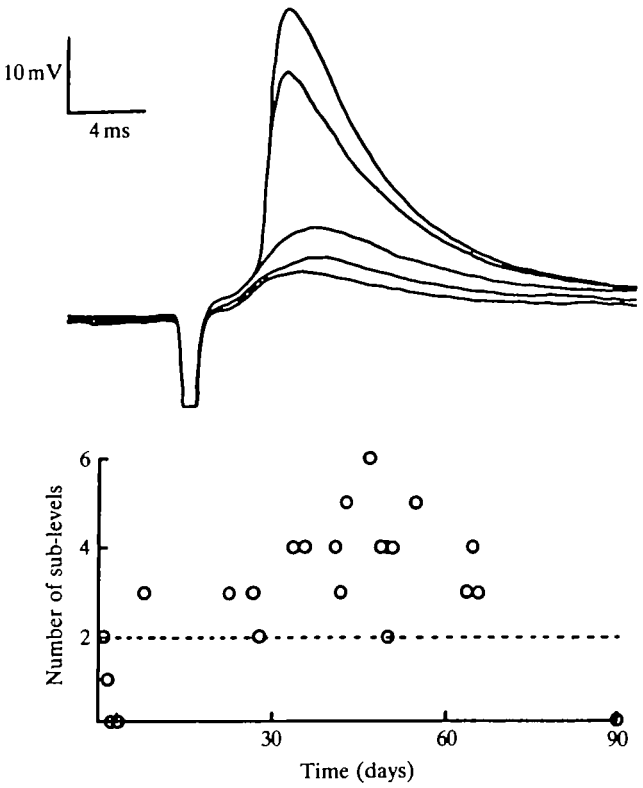

Fig. 6. Synaptic potentials recorded from the L_1 muscle after the nerve in segment II had been cut 6 weeks earlier. Top: the stimulus intensity to the nerve was gradually increased to recruit the axons innervating the L_1 muscle. Note that, now, five discrete steps of EPSPs were obtained, indicating that at least five axons innervate the L_1 muscle. Bottom: number of sub-levels seen in different preparations as a function of time after injection of pronase. (From I. Cohen, I. Parnas & J. Dudel, unpublished results.)

received new multiple innervation from its own segment. In two cases we have observed that axons from the posterior branch, from segment I, grew to cross the ventral midline and innervate the muscle on the contralateral side. It should be remembered that the muscles on the contralateral side had their complete, normal innervation. It seems that cutting of the nerve in segment II produces signals that allow innervation of muscle fibres by more than the two excitatory axons. In addition, muscles which still had their original normal innervation could be innervated by foreign nerves. Boundaries of innervation which were maintained so precisely in the controls appeared to relax and axons grew all over the muscle.

Removal of an entire neurone by intracellular injection of pronase has different effects from those seen after cutting the whole nerve. The difference in the signal or signals producing the different effects in the two treatments may be quantitative, and the removal of a single neurone may not be sufficient to elicit an effect. To test th possibility, all five axons innervating the deep abdominal extensor muscles should be removed by pronase injection and the effects of such a treatment on sprouting of the

posterior branch from segment I should be compared to those obtained after the cut. This experiment has not yet been done.

There are probably several independent signals that control the interactions between nerve cells and their common target. There appear to be signals that direct the nerves to grow and establish contacts and other signals that determine synaptic strength. While growth and sprouting have been the subjects of many investigations (for reviews see Purves, 1977; Purves & Lichtman, 1978), the quantitative analysis of synaptic strength has rarely been performed, largely because of the technical limitations of the system under investigation. Interactions between axons innervating the same target, releasing the same or different transmitters, seem to be more complex than hitherto thought.

Some of the responses described above may be classified as compensatory, i.e. compensating for the loss produced by the missing neurone. These include the expansion of the fields of innervation of the T and N neurones in the leech. The strengthening of the ExL_1 excitor after the removal of the Com.Ex. can still be looked upon as compensating for the missing strong innervation. In general, many of the changes described above are consistent with the concept of competition.

I greatly appreciate the critical reading of the manuscript by Dr H. Parnas and Dr H. Gainer. The secretarial help of Mrs Marie Morris and Josephine Schiaffino, the Fogarty International Center, is greatly appreciated. Supported by a grant from the USA–Israel Binational Fund and by the DFG, West Germany.

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