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FORMATION AND ELIMINATION OF FOREIGN SYNAPSES ON ADULT SALAMANDER MUSCLE

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SUMMARY

1. Synapses by flexor nerve were induced on denervated extensor muscle in adult salamander forelimbs. Excitatory potentials evoked by these 'foreign' synapses were at first small but increased to normal amplitude within several weeks, in the absence of correct nerve reinnervation.

2. Upon return of the correct nerve the efficacy of foreign synaptic transmission began to decline. The time of initiation of this decline correlated well with the resumption of correct nerve transmission. The suppression of foreign transmission involved a reduction in mean quantal content of transmitter release.

3. Suppression of foreign synapses was sufficiently thorough that most ceased transmitting entirely. Before reinnervation by the correct nerve 97 % of the extensor muscle fibres received functional foreign synapses while 4–6 months after correct nerve return only 35 % of the fibres retained foreign synapses, with weak transmission.

4. Two lines of evidence indicate that suppressed foreign synapses are lost from the muscle: (*a*) a second correct nerve lesion 6–8 months after the initial denervation produced no significant increase in the proportion of fibres with foreign transmission and (*b*) four muscles which showed complete suppression of foreign transmission were bathed in medium containing horseradish peroxidase (h.r.p.) and the correct nerve was stimulated repetitively. Subsequent histochemical staining for h.r.p. and examination of synapses by electron microscopy revealed that 94 % of the axon terminals had h.r.p. incorporated in vesicles. Thus at least that percentage of all identifiable synapses were from the correct nerve.

5. This ability to eliminate incorrect synapses in favour of correct ones is speculated to be a general characteristic of embryonic nervous systems, which in adulthood is retained by salamanders but lost by most other animals.

INTRODUCTION

Mature neurones and skeletal muscle fibres can be induced through denervation to accept innervation from foreign sources. Whether this foreign innervation remains functional when the correct nerve reinnervates its target is of interest from a developmental point of view. In mammalian skeletal muscle (Bernstein & Guth, 1961;

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Tonge, 1974; Frank, Jansen, Lomo & Westgaard, 1975; Brown, Jansen & Van Essen, 1976) foreign transmission continues unabated upon reinnervation by the correct nerve. The picture is less clear with the teleost fish. Using mostly behavioural criteria, Mark and his collaborators (Marotte & Mark, 1970*a*; Mark & Marotte, 1972) concluded that in cross-innervated extraocular muscles of the carp, the function of foreign synapses was suppressed upon reinnervation by the correct nerve. The absence of degenerating nerve terminals during correct reinnervation led them to propose further that suppressed foreign synapses are morphologically normal but silent (Marotte & Mark, 1970*b*; Mark, Marotte & Mart, 1972). Scott (1975) repeated and extended these experiments on the goldfish but concluded, in contrast, that suppression of foreign synapses did not occur. In addition, Frank & Jansen (1976) found no evidence of foreign synapse suppression with intracellular recording from gill muscles of the perch. A variety of studies in amphibia have indicated a selective preference for correct reinnervation of muscle fibres (Grimm, 1971; Cass, Sutton & Mark, 1973; Fangboner & Vanable, 1974; Hoh, 1971; Schmidt & Stefani, 1976). Moreover, Cass *et al.* (1973), who examined competition between correct and foreign nerve for reinnervation of adult newt muscle, concluded that the suppressed foreign synapses retained their morphological integrity.

In the present study, we have induced cross-innervation of a forelimb extensor muscle in the adult newt and looked for changes in properties of such 'foreign' synapses consequent to correct reinnervation. Our results confirm the finding of Cass *et al.* (1973) that foreign transmission is suppressed upon correct reinnervation. This suppression involves a decline in the quantal content of foreign transmitter release. However, both physiological and morphological evidence suggests that the suppressed synaptic terminals are eliminated from the muscle. Some of these findings have been briefly reported (Yip & Dennis, 1976).

METHODS

Surgical procedures. Adult newts, *Notopthalmus viridescens*, were anaesthetized with tricaine (1/1000 in Ringer solution). The forelimb flexor (humero-antibrachialis) muscle was excised and its nerve displaced laterally to the surface of the extensor (anconeus) muscle. The animals were returned to tanks of tap water and fed weekly with brine shrimp. Two weeks after the initial operation, the correct nerve to the anconeus muscle was crushed in one series of animals and resected in another. In some of these animals, the correct nerve was crushed or resected a second time 6–8 months after the initial nerve lesion.

Physiological recordings. At various times after the correct nerve lesion, the animal was pithed and the entire limb was removed with the third, fourth and fifth nerves. The spinal nerves were dissected through to the inferior and superior brachial nerves, which innervate respectively flexor and extensor musculature in the upper arm (see Grimm, 1971, for further description of innervation pattern). The limb was skinned, pinned on a Sylgard dish with a glass cover-slip bottom and the trunks of the two nerves were drawn into separate suction electrodes. All experiments were performed at room temperature in normal frog Ringer solution of the following composition (in m-mol/l.): NaCl, 120; KCl, 2; $CaCl_2$, 1·8; glucose, 10; HEPES buffer, 4 (pH 7·2).

Intracellular recordings were made using glass micropipettes filled with 4 M-K acetate and having resistances of 50–100 MΩ. The signals were fed through W.P. Instruments M4A amplifiers, 1X gain, and into a Tektronix D11 storage oscilloscope.

The quantal content of foreign nerve transmission was estimated both by the method of failures and from the coefficient of variation of responses (cf. del Castillo & Katz, 1954) evoked by stimulating 200 or more times at a frequency of 0·2 sec^{-1}. There was no significant difference between the values determined for individual fibres by these two methods.

Histological techniques. Active nerve terminals were labelled by stimulating uptake of horse-

radish peroxidase (h.r.p.) into synaptic vesicle membrane (Heuser & Reese, 1973). The muscle was pinned on Sylgard in a 0·5 ml. chamber and presoaked for an hour in h.r.p. (Worthington 10 mg/ml. normal frog Ringer solution). Equilibration of h.r.p. into the extracellular space was facilitated by squirting the solution on to the muscle with a Pasteur pipette. The nerve of interest was stimulated for 7 min with 10 Hz trains of 1 sec duration at 2 sec intervals. The preparation was then allowed to rest for 3 min. This pattern of stimulation and rest was repeated three times and the muscle allowed to rest in h.r.p. for an hour. The muscle then was extensively washed with frog Ringer solution containing 0-C^{2+}, 10 mM-$MgCl_2$ and 0·5 mM-EGTA. Solutions were changed throughout the wash. The muscle was fixed for 2 hr in a mixture 4 % paraformaldehyde and 2 % glutaraldehyde with 30 mM-HEPES (pH 7·2), left overnight in HEPES buffer, and chopped into slices of 50 μm width. Muscle slices were reacted histochemically with hydrogen peroxide and diaminobenzidine according to the method of Graham & Karnovsky (1966), post-fixed with osmium, embedded in Araldite (Ciba), and thin sections of synapses were obtained randomly throughout the muscles

RESULTS

Properties of normal anconeus fibres. Muscle fibres varied from 2 to 4 mm in length and 20–30 μm in diameter. The resting potentials were in the range of -90 to -110 mV. Spontaneous and evoked e.p.p.s. could be recorded at several sites along the muscle fibre (see also Lehouelleur & Chatelain, 1974 and Dennis, 1975). There was no evidence of electrical coupling between muscle fibres (Dennis, 1975).

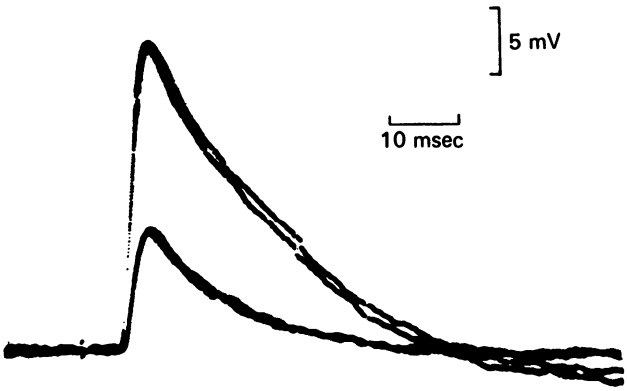
To check for multiple innervation, the intensity of stimulus impulses to the nerve was varied in small steps from below threshold to supramaximal. In 120 fibres sampled (eight muscles), twenty-four had a single synaptic input, sixty had two inputs and thirty-six received three inputs. Cholinesterase staining revealed that synapses were distributed at several sites along the length of individual fibres (unpublished observations). Such was also the pattern of innervation of the humero-antibrachialis, the antagonistic flexor muscle whose nerve was used in subsequent cross-innervation studies.

Muscles were checked for inappropriate synaptic inputs by independently stimulating the superior and inferior brachial nerves while recording from single fibres. Of 250 fibres sampled in nine muscles, none received foreign synaptic input.

Innervation of fibres by implanted foreign nerve. About 2 weeks after lesion of the correct nerve, foreign transmission began to appear in some muscle fibres. At this time repeated stimulation of the foreign nerve evoked e.p.p.s of low and variable amplitude, often with intermittent response failures. At longer times after correct nerve lesion the population of fibres innervated by the foreign nerve increased and was virtually complete within one month, provided the correct nerve was kept away. The foreign synaptic responses also increased in magnitude with time, and came to resemble those seen in normally innervated adult fibres; some muscle fibres were multiply-innervated by foreign axons, as indicated by distinct inputs of differing thresholds (Text-fig. 1) and in some the end-plate response was large enough to cause an action potential and twitch.

Decline in quantal content of foreign transmission. Approximately 2–4 weeks after the correct nerve was crushed or 6–8 weeks after it was resected, it began to reinnervate the muscle. The reformed correct synapses rapidly returned to their normal state of suprathreshold transmission (Text-fig. 2*A*). Conversely, foreign responses evoked subsequent to correct reinnervation were often of low amplitude (Text-fig. 2*B*) and failures were again frequent. In fibres in which foreign nerve stimulation was so

associated with intermittent release failures, the amplitudes of the evoked foreign responses were distributed as predicted by the failures method, using Poisson statistics (cf. del Castillo & Katz, 1954). Furthermore, when the amplitude histogram of these evoked foreign responses was compared to that of the m.e.p.p.s recorded at the same locus, the mean amplitudes of the spontaneous and the unitary evoked events were identical (Text-fig. 3), and were within the normal range (0·5–2·0 mV). Presumably the m.e.p.p.s. in such dually innervated fibres arose from transmitter release by correct as well as foreign terminals. Both the Poisson nature of foreign release and the identity in mean amplitude of spontaneous and foreign-evoked potentials indicate that the small foreign responses seen after correct reinnervation result from a low quantal content, rather than a reduction of quantal unit size or of transmitter sensitivity beneath foreign terminals.



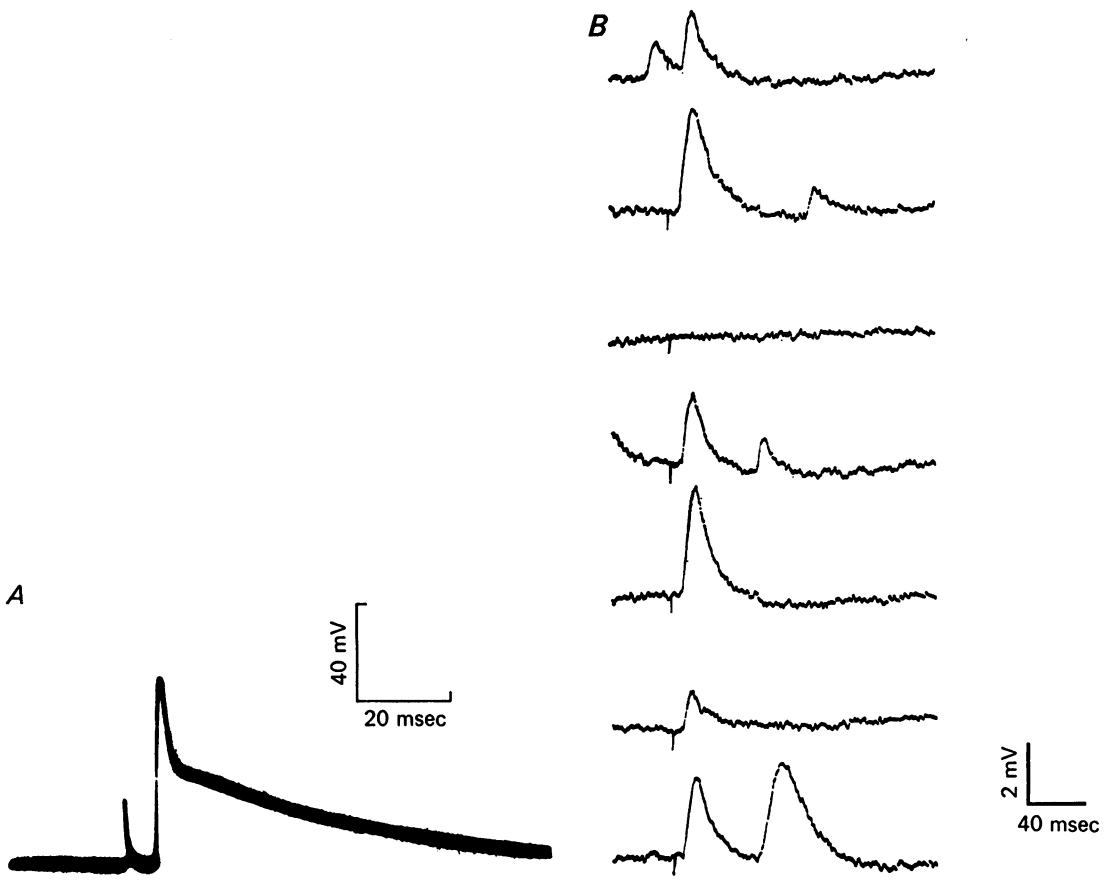
Text-fig. 1. Foreign synaptic responses 45 days after correct nerve resection. The fibre was innervated by two motoneurones as revealed by the increase in response amplitude with increase in the intensity of stimulus impulses to the nerve. Two responses were recorded at each stimulus intensity. No response was obtained from stimulation of the correct nerve.

The quantal content of foreign transmission was estimated in individual fibres at various times after the correct nerve lesion. In both series of animals studied, correct nerve resected and correct nerve crushed, the mean quantal content of foreign transmission declined with time, as illustrated in Text-fig. 4. The initiation of this decline was more rapid following nerve crush than after nerve resection, and was in both situations coincident with the onset of reinnervation by the correct nerve. This suggests that correct reinnervation and foreign suppression are causally related. It should be noted that the data which were used in deriving the mean quantal content at each time point in Text-fig. 4 were taken only from fibres which did show some foreign response. That is, fibres which showed no foreign response ($m = 0$) were not used in calculating the mean. Thus, these figures underestimate the decline in foreign synaptic transmission with time (see below).

At long times after the initial operation there was some regeneration of flexor muscle fibres and these were reinnervated by the flexor nerve. However, such regeneration occurred many weeks after the onset of suppression illustrated in Text-fig. 4. In two muscles where correct reinnervation by chance failed to occur or was sparse, the extent and efficacy of foreign transmission remained high, even 9 months after

correct nerve lesion. In both cases the foreign nerve had reinnervated its normal target (flexor) musculature as well.

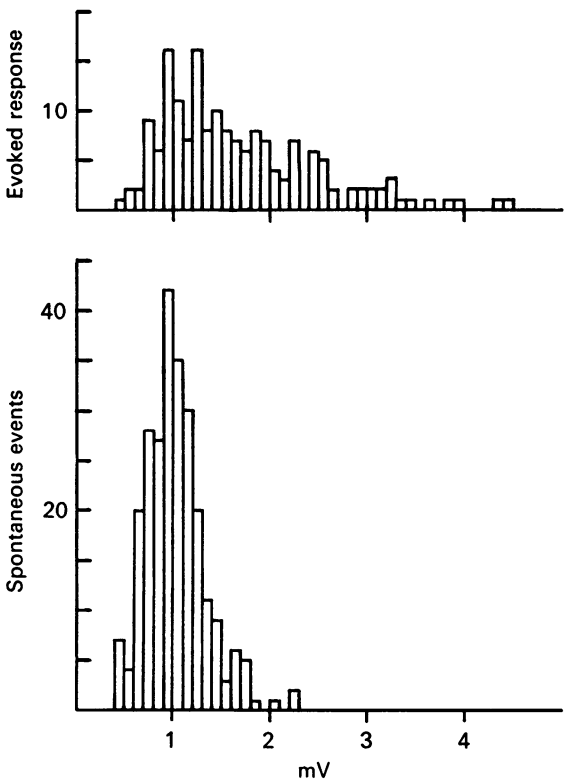
Decline in extent of foreign transmission. To get another index of the suppression of foreign synapses we asked how the extent of functional foreign innervation changed upon correct reinnervation. At various times after the correct nerve resection, intracellular recordings were made from single muscle fibres and the correct and foreign



Text-fig. 2. Recordings from a single fibre dually innervated by the correct and foreign nerves, 57 days after the correct nerve resection. *A*, large end-plate response with action potential superimposed evoked by correct nerve stimulation. *B*, consecutive responses to repeated maximal stimulation of the foreign nerve at a frequency of 1 sec^{-1}. A nerve stimulus of constant intensity occurs near the beginning of each sweep, as indicated by the artifact. With some stimuli, the foreign nerve failed to release transmitter (trace 3 from top). Spontaneous potentials appear randomly in several of the traces (1, 2, 4 and 7 from top).

nerves independently stimulated. In ten muscles examined 12–47 days after resection, $97{\cdot}3 \pm 1{\cdot}5\,\%$ of 215 fibres received foreign input, whereas in twenty-nine muscles 57–299 days after resection only 269 of 673 fibres ($39{\cdot}5 \pm 6{\cdot}5\,\%$) had functional foreign input. Thus the extent as well as the efficacy of foreign transmission declined upon reinnervation by the correct nerve, even though the suppression was not always complete.

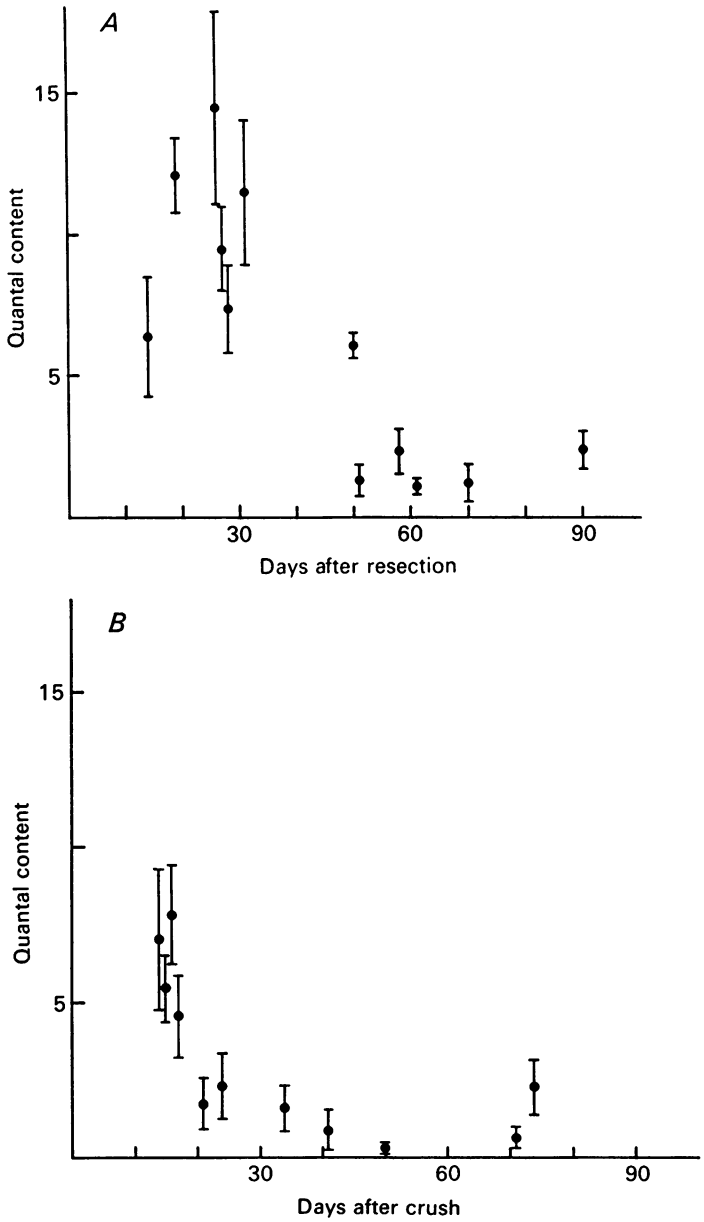
Second resection of correct nerve 6–8 months after the first. In the light of earlier claims of morphological integrity of suppressed foreign synapses (Mark *et al.* 1972; Cass *et al.* 1973) it was of interest to know the fate of such synapses in our system. To determine whether suppressed foreign synapses could be rapidly reactivated upon loss of correct innervation we resected the correct nerve a second time long after the correct nerve had regenerated (6–8 months after the first resection). In seventeen muscles (383 fibres) sampled 2–20 days after a second correct nerve resection, an average of



Text-fig. 3. Amplitude histograms of spontaneous (bottom) and foreign evoked (top) responses recorded from a fibre 50 days after correct nerve resection. The foreign nerve was stimulated 294 times with 114 failures. The mean amplitude of the foreign evoked responses = 1·05 mV whilst that of the spontaneous events = 1·08 mV. Stimulation of the correct nerve resulted in an action potential. Medium contained 1·8 mM-Ca^{2+}.

$36{\cdot}2 \pm 9{\cdot}8\,\%$ of the fibres had functional foreign inputs. By comparison, in seventeen control muscles (453 fibres), of the same experimental age whose correct nerve had been resected only once 6–8 months earlier, $35{\cdot}1 \pm 7{\cdot}8\,\%$ of the fibres still had some functional foreign innervation. The large standard error of these values reflects the considerable variability encountered from muscle to muscle; in some, no foreign transmission could be detected. The end-plate responses to foreign nerve stimulation were similar in both sets of animals, typically of 1 to several mV amplitude. Thus, foreign transmission did not increase in extent or efficacy, up to 20 days after a second correct nerve resection, at long times after correct reinnervation. Since the

initial development of foreign transmission took place in 14–18 days (Text-fig. 4), these results suggest that foreign synapses are not available for reactivation at long times after their suppression. In an initial note (Yip & Dennis, 1976) we erroneously concluded that foreign transmission did increase after a second correct nerve lesion.



Text-fig. 4. Mean quantal content of foreign nerve release plotted as a function of time after the correct nerve was resected (*A*) and crushed (*B*). Each point is the average of the quantal contents measured in six to ten fibres. Each vertical bar represents the standard error of the mean. Fibres which showed no foreign responses were not used in calculating the mean. Note that the quantal content of foreign transmission becomes higher and remains so longer when the correct nerve is resected.

This error resulted from failure to sample a sufficiently large population of experimental and control muscle fibres.

H.r.p. labelling of active nerve terminals. The best evidence for the existence of morphologically normal but functionless synapses would be a demonstration of their presence by electron microscopy. For this purpose, one would like to be able to distinguish correct from foreign synapses. Cutting the correct nerve so as to use its degeneration products to distinguish it from foreign terminals (Mark *et al.* 1972) is not satisfactory because sprouting of foreign axons might be induced. Therefore, to label correct nerve terminals we made use of the observation of Heuser & Reese (1973) that h.r.p. is taken up by active nerve terminals during vesicle recycling.

To assure ourselves that the technique was applicable in this system, two normal muscles were prepared for h.r.p. experiments, and the correct nerve stimulated as described in Methods. In sixty-one end-plates studied, 95 % were positively labelled as judged by the presence of h.r.p. reaction product on their vesicles. In many of these terminals, more than 50 % of the vesicles were labelled (Pl. 1*A*). This was clearly different from background uptake (Pl. 1*B*), determined in another muscle which was soaked in h.r.p. but not stimulated; here only one or a few vesicles per terminal were labelled.

Normal nerve terminals were characterized by an abundance of clear vesicles, a few with dense cores, mitochondria, occasional neurofilaments, and a thin layer of Schwann cell cytoplasm over their outer surfaces. Terminals were closely apposed to muscle membrane which in some synapses showed well established subsynaptic folds. Their ultrastructure was similar to that reported by Lentz (1969) for the mature newt synapse. Most of the synapses showed only a single nerve ending. Occasionally two or three axon terminals were found apposing a single end-plate, but we do not know whether these were branches of the same axon or were terminals from different motoneurones.

Four cross-innervated muscles which showed no sign of remaining foreign transmission by intracellular recording (125–274 days after correct resection) were prepared for h.r.p. uptake. After correct nerve stimulation 94 ± 3 % of 293 terminals showed positive h.r.p. labelling of their vesicles. To ensure that we were not missing foreign terminals located in a restricted region of the muscle, samples of synapses were taken randomly throughout the muscle; in one case, the long axis of a muscle was cut into three pieces, each was re-embedded and samples taken from each segment. This extent of labelling is like that observed in control experiments on normal muscles (95 %) and suggests that few if any of the synapses on these muscles are foreign. In the absence of physiological signs of foreign innervation following correct reinnervation, foreign terminals are not physically present.

We also studied h.r.p. uptake in two muscles which did not show complete suppression of foreign transmission, 225 and 299 days after correct nerve resection. In these muscles 30 % of the fibres sampled had residual foreign transmission. Upon correct nerve stimulation in h.r.p., 68 % of 150 terminals were labelled, 32 % were not. There was no obvious difference in ultrastructure between labelled and unlabelled terminals.

In one muscle, which by intracellular recording showed no sign of foreign response, 228 days after the initial correct resection the foreign nerve was stimulated in the

presence of h.r.p. Of twenty-seven synapses examined all showed no more than background uptake. This negative result served as an additional control against background uptake by unstimulated (in this case, correct) terminals and further supported the conclusion that suppressed foreign terminals are eliminated.

It was of interest to know whether suppression of foreign transmission required direct contact between correct and foreign terminals, especially during the early stages of correct reinnervation when profiles of several terminals are often seen at single end-plates. With this in mind, h.r.p. labelling of the correct nerve was attempted in several muscles at a time when correct reinnervation was in progress. Some of the terminals were labelled and others were not. However, it was not certain that all of these unlabelled terminals were foreign since some of the newly developing correct synapses would be expected not to transmit (Dennis, 1975) due to failure of action potentials to propagate into their terminals (Dennis & Miledi, 1974). Such terminals would not take up h.r.p. To check this possibility we simply denervated and awaited reinnervation of an anconeus muscle, without introducing a foreign nerve. At the time of ongoing reinnervation, the muscle was soaked in h.r.p. and its nerve stimulated as above. Upon examination in EM we here too found multiple axon profiles at single end-plates with some terminals labelled while others were not. Thus, the h.r.p. labelling technique did not permit us to unequivocally distinguish correct from foreign terminals at the time when correct reinnervation was in progress.

DISCUSSION

These results indicate that in adult newt muscle foreign synapses are suppressed as a consequence of correct reinnervation. This suppression involves a decline in the quantal content of transmitter released, with no change in the size of the quantal packets or in the post-synaptic transmitter sensitivity. In most instances this reduction of transmission went to completion and the foreign terminals were eliminated: 35 % of the fibres examined at long times (6–12 months) after correct reinnervation did retain some foreign synaptic function. However, transmission on these fibres too was reduced in that the evoked foreign responses were only a few millivolts in amplitude, in contrast to the suprathreshold foreign responses seen prior to correct reinnervation. Our results do not resolve whether the observed decline in quantal content occurs prior to terminal elimination or whether that decline occurs *as* the terminals are lost.

One would like to know the nature of the signal which initiates the loss of foreign transmission. One possibility is that there is direct contact between foreign and correct terminals which somehow triggers foreign nerve withdrawal. Experiments on errors of innervation made spontaneously during salamander limb regeneration (J. W. Yip & M. J. Dennis, unpublished) indicate that correct and incorrect synapses occur at separate sites on single muscle fibres, and that incorrect synapses are suppressed. This suggests the alternative possibility, that the signal for withdrawal of incorrect synapses is mediated by the target muscle fibre. Such a signal could conceivably involve either a chemical factor released by the target or the pattern of electrical activity of the muscle. Further investigation is necessary before speculation about this mechanism is warranted.

Our conclusion that suppressed synapses are eliminated from the muscle is in striking contrast to the claim of Cass *et al.* (1973) that suppressed synapses are morphologically normal. Two lines of evidence led Mark and his collaborators to believe that there were silent synapses in cross-innervated muscles: (1) the absence of degenerating synapses during correct reinnervation, and (2) the presence of both normal and degenerating synapses several days after a second correct nerve cut. These observations may be explained without invoking the presence of 'silent' synapses. First, upon correct reinnervation, foreign terminals may retract without leaving recognizable degeneration products. Such a mechanism has been indicated in the elimination of polyneuronal innervation of neonatal rat skeletal muscle, in that no morphological signs of synapse degeneration were seen at a time when the number of transmitting synapses was being reduced (Korneliussen & Jansen, 1976). Secondly, the morphologically normal terminals in cross-innervated goldfish fibres observed several days after the correct nerve was cut (Mark *et al.* 1972) may have been those of *functional* foreign synapses: the behavioural test used in that study was not sufficiently sensitive to reveal subthreshold synaptic transmission. More recently, Scott (1975) has questioned whether suppression of foreign transmission occurs at all in goldfish extraocular muscle.

Only 36 % of the muscle fibres examined had functional foreign innervation soon after a second correct nerve resection, 6–8 months after the first. Thus, a second correct nerve lesion produced no significant increase over the 35 % residual foreign transmission seen without a second correct denervation, which was quite distinct from the complete foreign innervation seen after the first correct resection. This indicates that suppressed synapses are not intact. The fact that the residual functional foreign terminals do not sprout to innervate all of the muscle fibres following the second degeneration of correct terminals is somewhat surprising. This may result because the remaining foreign axons are extended beyond their normal scope of innervation and, as a consequence, are not liable to further extension (cf. Brown *et al.* 1976). Such a phenomenon does not, however, seem to be the primary cause of foreign suppression; suppression was well correlated with the time of correct reinnervation, varying according to whether the correct nerve was crushed or resected, regardless of the regeneration and subsequent reinnervation of the normal target of the foreign nerve (m. humeroantibrachialis). Furthermore, in two muscles the correct nerve failed to reinnervate and in those the extent and efficacy of foreign transmission remained high even though the normal target of the foreign nerve was well regenerated and innervated.

Unlike the situation we have described here adult mammalian muscle appears unable to reject foreign innervation once it has been established (Bernstein & Guth, 1961; Frank *et al.* 1975; Brown *et al.* 1976). Urodele amphibia retain in adulthood several embryonic characteristics and in this category perhaps belongs the ability to distinguish foreign from correct synaptic inputs and to suppress the foreign. Consistent with this idea is recent evidence (Yip & Dennis, unpublished) that during newt limb regeneration inappropriate neuromuscular synapses sometimes form but are subsequently removed in favour of the correct ones. Some selectivity in retention of synapses also occurs in adult anuran amphibia. During reinnervation of frog and toad muscles, slow muscle fibres initially receive input from fast motor axons (Schmidt & Stefani, 1976), but this is subsequently replaced by the appropriate

slow innervation (Hoh, 1971; Schmidt & Stefani, 1976). Following foreign innervation of the pectoralis muscle of *Xenopus* the original nerve re-establishes synapses, which increase with time in quantal content of evoked transmitter release. When such a junction develops in the same synaptic area as occupied by a foreign axon, the foreign terminal ceases to increase in transmitter output (Haimann, Mallart & Zilber-Gachelin, 1976). This competitive interaction does not occur when correct and foreign terminals are separated by several millimetres. Also upon innervation of ectopically placed frog muscle by two incorrect nerves, there occurs a sorting out of fields such that each nerve synapses with some of the fibres yet with little overlap in the territory of the other nerve (Grinnell, Rheuben & Letinsky, 1977).

The ability of a target cell to distinguish between a variety of presynaptic inputs and eliminate some would be of obvious utility in development and moulding of the central nervous system. The lack of suppression of inappropriate synapses experimentally induced on cells of adult mammals probably results from a loss during maturation of flexibility present in the developing nervous system; such a loss of plasticity with age has been clearly shown in the mammalian visual system (Hubel & Wiesel, 1970). We feel that further examination of normal embryonic development will reveal a mechanism for enhancing the precision of excitable cell interaction such as that described here.

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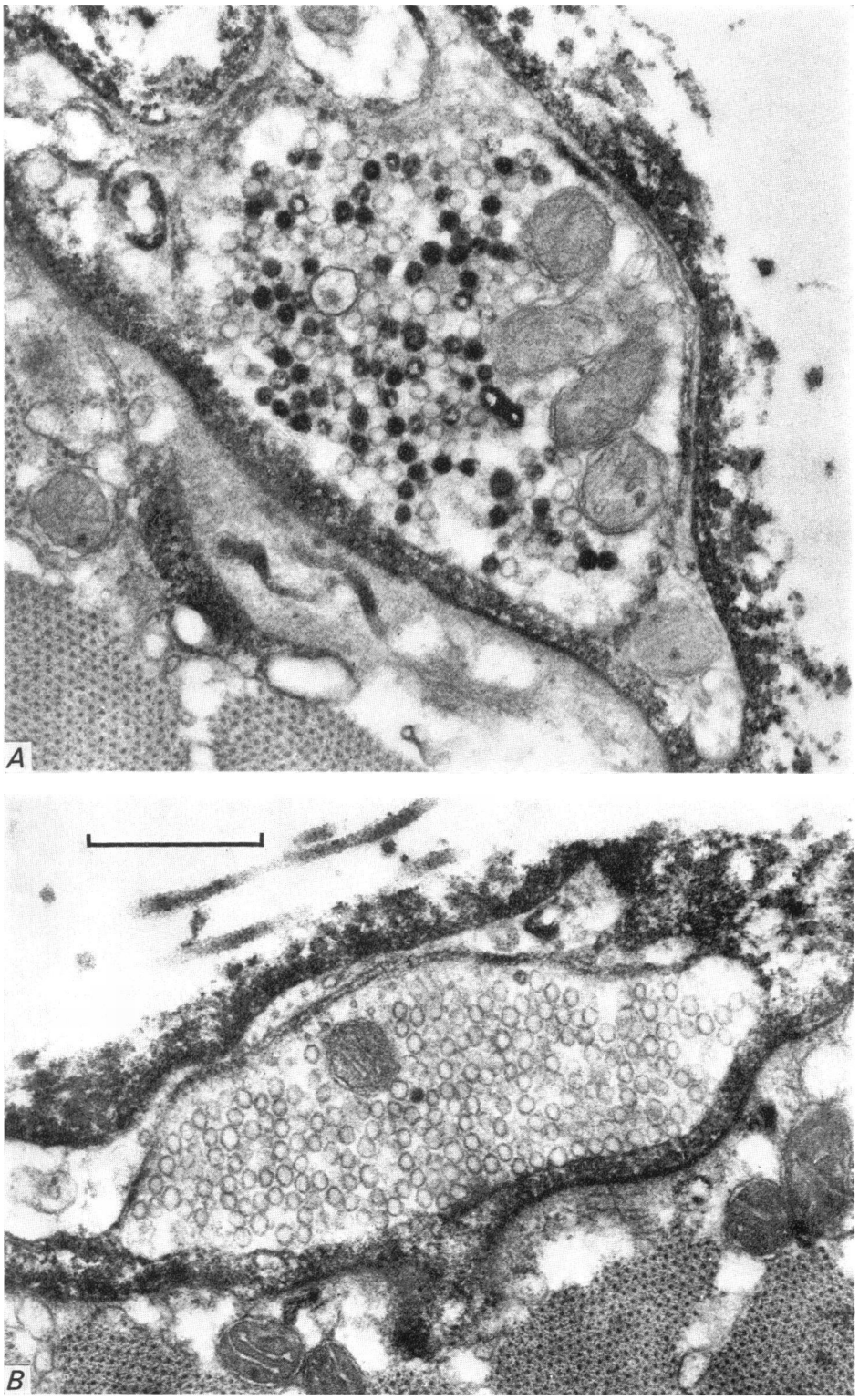
EXPLANATION OF PLATE

PLATE 1

A, cross-section of a normal anconeus end-plate stimulated for h.r.p. uptake as described in text. The nerve terminal, surrounded by a thin layer of Schwann cell cytoplasm, contains synaptic vesicles and a few mitochondria. 48% of the vesicles contain the electron-dense peroxidase reaction product, indicating that they contain h.r.p.

B, cross-section of a normal anconeus terminal soaked in h.r.p. but not stimulated, to show background uptake. Calibration bar, 0·5 μm.


A
B