Electrophysiological Experiments on the Mechanism and Accuracy of Neuromuscular Specificity in the Axolotl [and Discussion]

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Electrophysiological experiments on the mechanism and accuracy of neuromuscular specificity in the axolotl

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[Plate 1]

The supracoracoideus muscle of the axolotl shoulder girdle is innervated by two nerves, the supracoracoideus nerve (SC) supplying most of the muscle and the posterior supracoracoideus (PSC) supplying the posterior corner. All the muscle fibres are multiply innervated and at the border between the two innervations many muscle fibres, when penetrated by a microelectrode, show junction potentials from both nerves. In such cases one junction potential is often very small, below the threshold for exciting muscle contraction, the other large and effective at exciting the muscle. If the SC nerve is cut, the territory of the PSC nerve expands over several weeks. Upon regrowth of the cut nerve it reinnervates its old muscle fibres and removes the previous foreign innervation, the borderline between the two nerve territories being established exactly as before. This depends upon two processes, sprouting of nerves and a competitive repression of transmission from nerves ending on foreign muscle fibres.

1. INTRODUCTION

It has been known since the work of Paul Weiss (1922) that the tailed amphibians show a remarkable ability to adapt to surgical damage of their limbs or of their motor innervation. Reinnervation of regenerated or grafted limbs always results in well timed activation of limb muscles, fitting in with the natural sequence of contraction shown by the muscles of the normal legs. The site of such adaptive ability, Weiss (1927) suggested, was the spinal cord, which could alter the discharge patterns of motoneurons, depending upon which muscles a comparatively undirected growth of their axons took them to. More recent experiments on this and related phenomena have made it appear unlikely that the reflex connections of motoneurons show the required plasticity (Sperry 1945; Mark 1969). Further work was required to check on the other explanation – that growing motoneuronal axons can recognize and reconnect specifically with their appropriate muscles. (Marotte & Mark 1970*a*, *b*; Mark & Marotte 1972; Mark, Marotte & Mart 1972; Mark 1974).

In axolotls, motor nerves that have been cut and misdirected into the hind limb reform neuromuscular connections so as to restore the original segmental pattern of motor innervation (Cass & Mark 1975). When one of the 3 or 4 segmental nerves that normally supply the leg is cut, the muscles it previously controlled become innervated by the remaining nerves (Aguilar, Bisby, Cooper & Diamond 1973; Cass, Sutton & Mark 1973; Stirling 1973). Connections from foreign nerves are therefore not prohibited, but when the full complement of nerve fibres

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is allowed to grow together into the limb, some process sorts out where the fibres from each nerve root form their fully functional connections with the muscles. Peripheral, rather than central, selectivity in the formation of connections it seems might play the major role in the return of coordinated movement to reinnervated legs.

Since specific reconnection must depend on the exchange of developmental information between motor nerves and muscle fibres, one question that arises is how detailed that information might be. There is evidence that regenerating motor nerves in many species recognize the appropriate type of muscle, forming connections more readily in those of the kind and contraction speed they previously commanded (see, for example, Hoh 1975). Grimm's (1971) results in the axolotl forelimb suggest that the spatial selectivity of innervation in these animals extends to the level of anatomically defined muscles. But could it be more precise, extending to the level of regions of a muscle or perhaps to individual muscle fibres?

A second question concerns the mechanisms of selective innervation. Competition between motoneurones to innervate muscles appears to be involved, but on what processes of growth or function of neuromuscular terminals does the competitive process act?

Using electrophysiological techniques we have been looking at reinnervation of supracoracoideus muscle of the axolotl shoulder girdle. Our findings go some way towards answering the question of the level of spatial selectivity and give some suggestions as to likely mechanisms underlying the process.

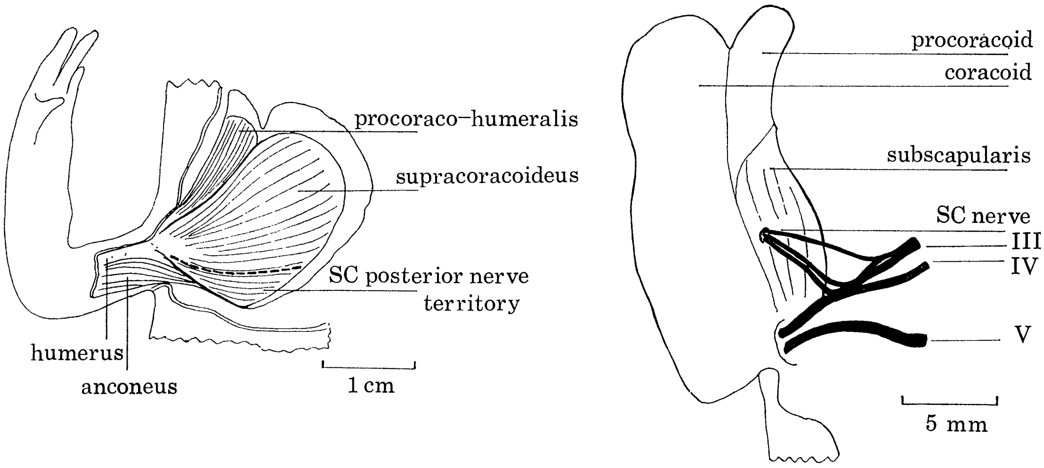

FIGURE 1. (*a*) A drawing showing the location of the supracoracoideus muscle on the ventral aspect of the axolotl shoulder girdle. Anterior to the top of the drawing. The dotted line shows the approximate position of the anterior border of the territory of the posterior supracoracoideus nerve in the muscle. (*b*) A drawing of the structures on the inner surface of the coracoid as they are seen when it is reflected back to expose the SC nerve, i.e. as the right hand edge of figure 1*a* is lifted up to expose the structures on its inner surface.

2. METHODS

(*a*) *Animals*

Larval axolotls, 10–15 cm in length were kept in individual aquaria and maintained on a diet of worms and beef liver.

(*b*) *Preparation*

The supracoracoideus muscle of the axolotl shoulder is easily accessible, lying just beneath the skin on the ventral surface of the thorax. It is composed of a thin fan-shaped layer of fibres which take their origin from the cartilaginous plate of the coracoid and insert into the humerus (figure 1*a*). There are normally two peripheral nerves supplying the muscle. The supracoracoideus (SC) nerve that innervates most of the overlying muscle is composed of nerve branches from the 3rd and 4th spinal nerve roots which pass ventrally through a small hole between the procoracoid and the coracoid (see figure 1*b*). The posterior supracoracoideus (posterior SC) nerve, also composed of branches from the 3rd and 4th spinal nerves, innervates only the posterior corner of the muscle. Functionally, the two nerves are separate. Stimulation of the SC nerve pulls the humerus medially towards the ventral midline and would therefore assist in raising the animal from the ground, whereas stimulation of the post SC nerve initiates retraction of the humerus.

(*c*) *Operations*

Axolotls were anaesthetized by immersion in 2.5 % urethane in water for 10–15 min. A ventral incision was made along the midline between the forelimbs to expose the edge of the coracoid. After removing the connective tissue, the plate of the coracoid was lifted up and separated from the underlying body wall to expose the SC nerve which was crushed, cut or a section removed as close to the spinal nerves as possible. The coracoid was returned to its original position, the skin sutured and mercurochrome painted over the surrounding area to prevent infection. The operation took about 20 min and the skin of the animal was kept moist during this time. Twenty eight animals were operated, of which 17 survived for analysis.

(*d*) *Electrophysiology*

Each axolotl was anaesthetized, pithed, and the SC muscle exposed. The body wall musculature was removed to reveal the brachial plexus and the SC nerve. The 3rd, 4th and 5th spinal roots of the plexus were cut close to the spinal cord and dissected free. The SC muscle, attached to the coracoid and the humerus, was taken out together with the spinal nerves and mounted in a dish of amphibian Ringer. The SC nerve was threaded through the subscapularis muscle (figure 1*b*) and drawn into a suction electrode. The posterior SC nerve was stimulated through suction electrodes applied to the 3rd and 4th spinal nerves. Short trains of square current pulses, usually 3–5 stimuli, were applied every second. Each stimulus was of 0.5 ms duration and the interval between stimuli was approximately 16 ms. Endplate activity in response to nerve stimulation was recorded with an intracellular glass microelectrode, 10–60 MΩ, filled with 3 M KCl. Conventional recording apparatus was used to display electrical events on a cathode ray oscilloscope and a camera used to film the traces. During the first few experiments, neuromuscular transmission was reduced with Mg^{2+} but this became unnecessary with improved technique.

(*e*) *Mapping*

A drawing tube (× 12) was used to outline the SC muscle and mark in any obvious features such as blood vessels and pigment spots, features that were used to locate accurately the position of the microelectrode in the muscle. The microelectrode was moved across the muscle sampling the innervation of individual muscle fibres. For every successful penetration of a fibre, the SC and the posterior SC nerve were stimulated in turn and the type of activity evoked noted. The location was plotted on the drawing of the muscle. Trains of stimuli were applied to the nerve and the edge of the contracting area of muscle observed. This border was compared with that resulting from estimating the extent of innervation by junction potentials recorded with the microelectrode. The operated side of the animal was always mapped first. The unoperated control side was left overnight in Ringer at 4 °C and mapped the following day.

(*f*) *Histology*

Frozen sections of the muscle, made tangential to the coracoid so that almost the whole muscle was included in the central section, were stained by Coupland & Holmes' (1957) method for cholinesterase.

3. RESULTS

(*a*) *Histology*

The muscle fibres of the supracoracoideus are arranged in a radial manner, mainly taking origin from the margin of the plate of the coracoid and coming together to insert into the humerus.

When stained for cholinesterase, the muscle is seen to be densely innervated by bands of nerve endings which run in arcs across the line of the muscle fibres, each arc separated by some 100–200 μm (figure 2, plate 1). Individual nerve endings are elongated in the direction of the muscle fibres. The stained deposit is 1–2 μm wide and anything from 5 to 200 μm long. In some less heavily stained endings the deposit appears as a series of connected dots 1–2 μm in diameter. Where muscle fibres can be followed they are seen to receive a terminal from each band of nerve endings. The pattern of innervation is uniform from the anterior to the posterior border of the muscle. Nerve endings in different parts of the muscle show no obvious morphological features, and no changes in the distribution of the lengths of the endings.

(*b*) *Electrophysiological mapping of innervation*

Of the two peripheral sources of innervation of the muscle, the main SC nerve contains a variable contribution of fibres from the 3rd and 4th spinal roots. Stimulation of this nerve causes contraction of the entire muscle with the exception of the posterior edge. The posterior SC nerve is mainly composed of fibres from the 4th spinal nerve but sometimes there is a contribution from root 3. When the SC nerve is cut and the 3rd and 4th roots are stimulated, which is the method we used for exciting the posterior SC nerve, there is contraction of the postero-lateral corner of the muscle only. Although the territory of innervation of the posterior SC nerve overlaps extensively that of the SC nerve, the anterior border of this territory is sharp and can be clearly seen when watching muscle contraction through a dissecting microscope. The posterior border of the overlapping territory of the SC nerve, within the territory of the posterior SC nerve, is less definite.

With an intracellular microelectrode, two kinds of muscle fibres can be distinguished by their response to stimulation of the nerves. One kind gives rise to junction potentials which facilitate with repetitive stimulation until the threshold for an action potential is reached. In others one records large (20 mV) junction potentials which also facilitate with repetitive stimulation but not action potentials. Such activity does cause muscle contraction.

In the area of overlap of innervations, and occurring on both kinds of muscle fibres, there is another class of junctional activity. These are small junction potentials, whose amplitude seldom exceeds 3 mV even with maximal stimulation of the nerve and which do not facilitate significantly with repetitive stimulation. The examples of small junctional activity demonstrated in figure 3 are responses to maximal nerve stimulation recorded while maintaining a steady membrane potential of at least 70 mV. Because of their small amplitude and their lack of

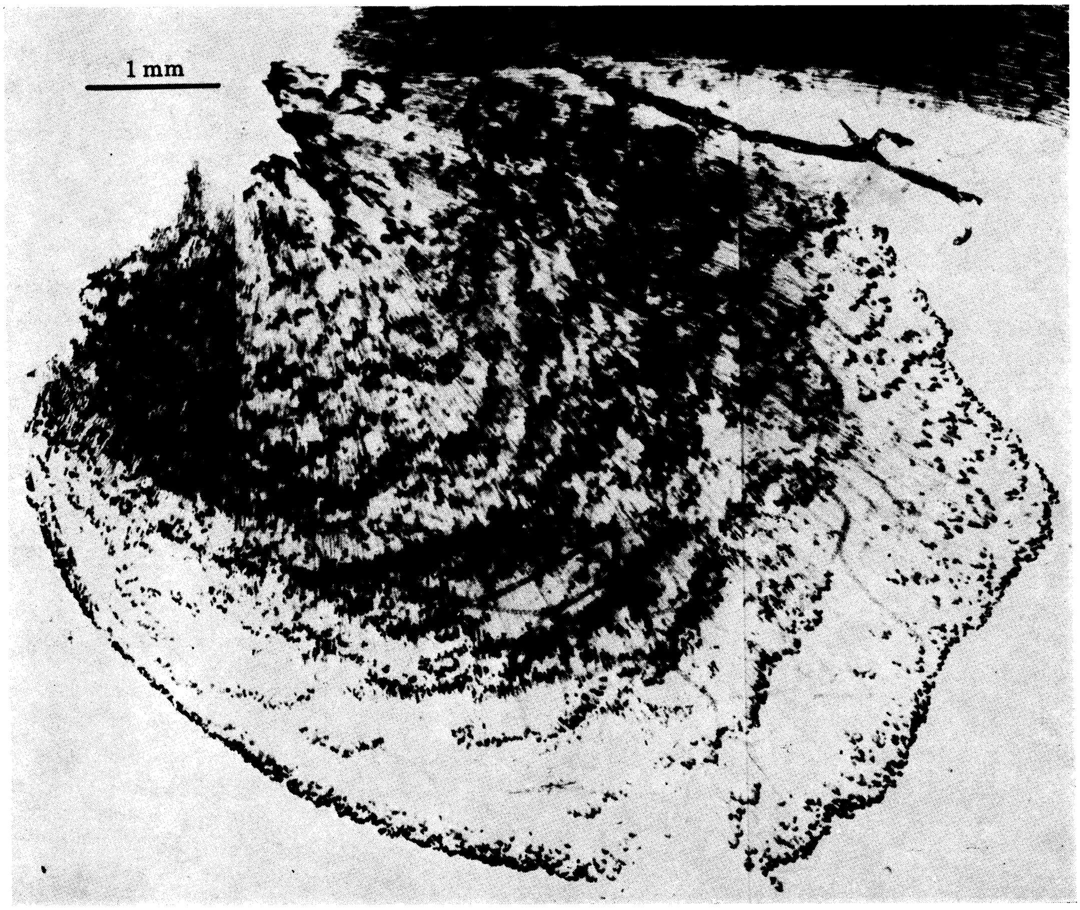

FIGURE 2. A photomontage of a section of the whole of the supracoracoideus muscle stained for cholinesterase to show nerve endings. Note how they are arranged in arcs across the line of the muscle fibres.

temporal facilitation, small junction potentials must by themselves be ineffective in causing muscle contraction.

In the area of overlap between the adjacent nerve territories, endplate activity from both nerves is often present in the same muscle fibre. Occasionally both nerves elicit action potentials, but usually there is an action potential from one nerve and a small junction potential from the other. At no time were two small junction potentials recorded as a result of stimulation of different nerves with endings on the same muscle fibre. A small junction potential from one nerve was inevitably accompanied by an effective connection from the other nerve, one that set up an action potential or a large junction potential (figure 4). The latency of junctional activity was from 1 to 3 ms throughout the muscle.

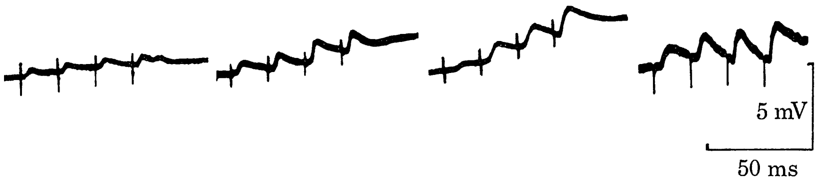

Figure 3. Examples of small junction potentials. Photographs were taken on moving film so the baseline slopes up to the right. Note the low amplitude and relative lack of temporal facilitation.

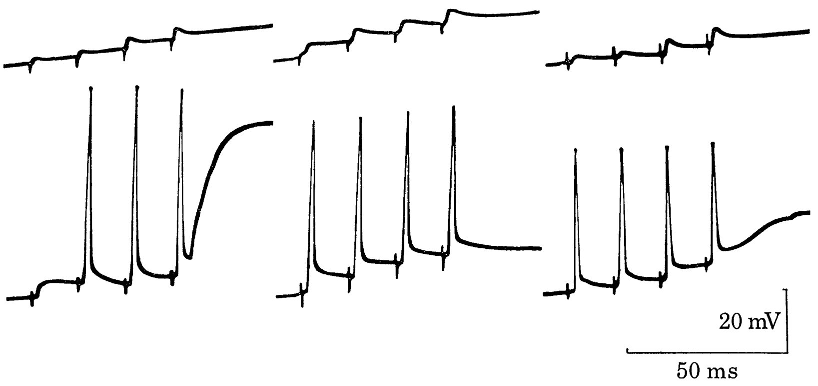

Figure 4. Pairs of records from 3 muscle fibres in which the upper shows small junction potentials elicited by stimulation of one nerve and the lower action potentials from stimulation of the other nerve. Photographs were taken on moving film so all base lines slope up to the right.

A map of the precise territory of each nerve in the muscle can be made by recording endplate activity with an intracellular microelectrode in response to repetitive nerve stimulation (4 shocks at 16 ms intervals). In figure 5 only the posterior corner of a normal muscle, the area of overlap, is outlined with symbols indicating the nature of the innervation recorded in various positions. The dashed line, which follows the direction of the muscle fibres in this part of the muscle, represents the boundary of the contracting area observed when the posterior nerve was stimulated repetitively. The dotted line indicates the relative position of the same border in the muscle on the opposite side of the animal. The wavy lines are patterns of pigmented blood vessels used in making the map. A fair degree of symmetry in the size of the smaller nerve field can normally be assumed.

Action potentials and large amplitude junction potentials can be recorded in response to stimulation of the posterior SC nerve throughout the posterior corner region. Such activity becomes rare towards the border but the junction potentials of very small amplitude are found. A similar pattern of innervation is found when the junctional activity from the main SC nerve is mapped. In its own territory and where it overlaps the territory of the posterior nerve, action potentials and large junction potentials are readily recorded and small junction potentials are found towards the posterior edge of the muscle.

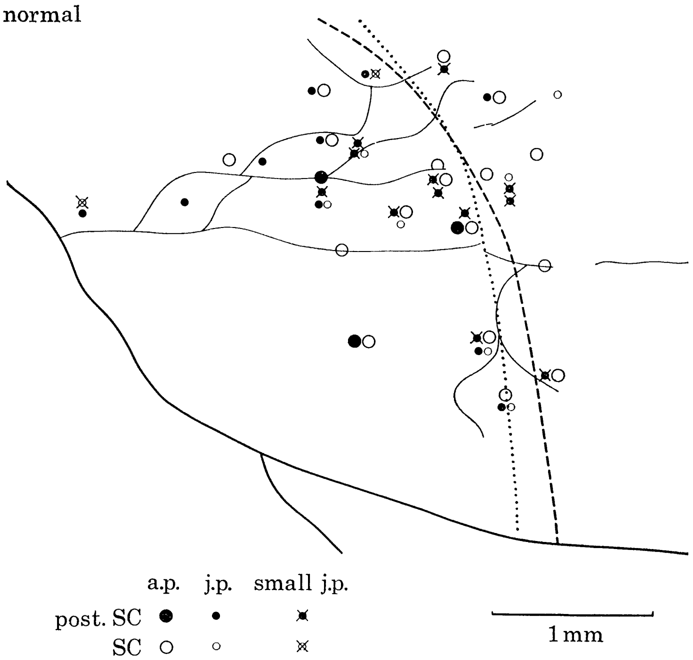

Figure 5. A detailed map of the location of junctional activity recorded following stimulation of the SC nerve (○) or the posterior SC nerve (●) in the region of the border between the two territories. Symbols side-by-side indicate activity recorded from the same fibre. Large circles are action potentials, small circles junction potentials. Symbols with crosses through them represent small junction potentials (see figures 3 and 4). The dashed line shows the anterior contraction border of the territory of the posterior SC nerve, the dotted line the position of the border in the contralateral muscle.

(*c*) *Denervation and reinnervation*

Changes in the innervation of the SC muscle were investigated at various times after crushing the SC nerve. There were no obvious behavioural effects of the operation – axolotls were able to swim with full use of their forelimbs. Six animals were used in this series. They were killed and their muscle innervation mapped at equal intervals of from 1 to 6 weeks post-operatively. Figure 6, shows examples of the effect of the operation on the size of the nerve fields. The top diagram in each pair is the outline of the operated muscle; that underneath is the opposite unoperated control drawn as its mirror image to facilitate comparison with the experimental side. The solid and open circles represent activity recorded from the posterior SC and the SC nerves respectively. The number of symbols on each map indicate the number of points sampled and not the density or strength of innervation. Symbols which overlap represent activity from the two nerves recorded in the same muscle fibre. The solid line represents the edge of the contracting area seen following repetitive stimulation of the posterior SC nerve. The dashed line is the border of the contracting area stimulated by the SC nerve. When no dashed line is shown the SC nerve territory extended over almost the whole muscle. Whereas the border of the posterior SC nerve territory was usually sharp, the border of the SC nerve at the posterior edge of the muscle was often not easy to define by watching muscle contraction.

Up to 2 weeks after crushing the main SC nerve, little change was detected in the size of the posterior SC nerve field. The SC nerve had begun to reinnervate its own muscle fibres (the dashed line represents the extent of its regeneration), but there was still a considerable patch of denervated muscle between the two innervated areas. After 3 weeks, there was definite spread of innervation from the posterior SC nerve into the denervated territory. Longer latency small junction potentials were recorded from the border region. After 4 weeks the crushed nerve had regenerated and reinnervated its own muscle fibres to the extent that there was a small amount of overlap with innervation of the posterior SC nerve. The posterior SC nerve field had been

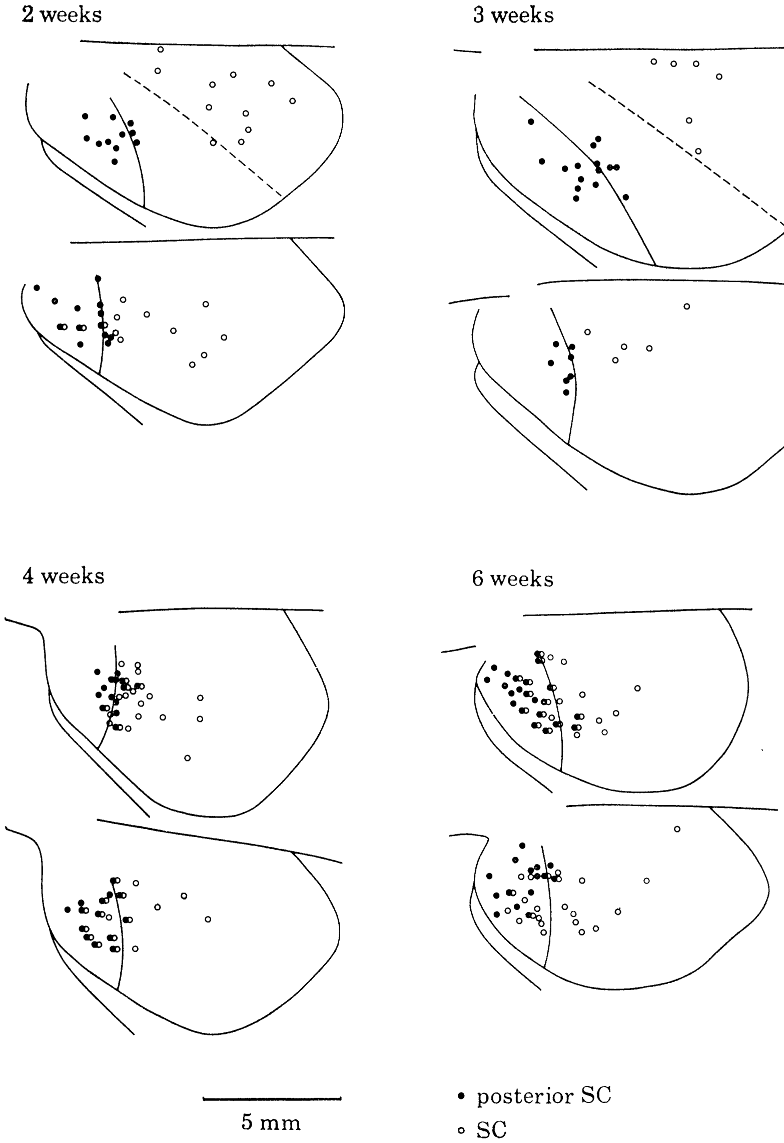

FIGURE 6. Patterns of innervation at various times after crushing the SC nerve. Symbols represent points at which junctional activity was recorded. ●, ——, activity and the contractions border elicited from the posterior SC nerve, ○, – – –, that from the SC nerve. For each pair the upper diagram is from the operated side, the lower from the opposite normal side drawn as a mirror image of itself to facilitate comparison of the pairs. Comparing the two sides in each case note the expansion of the territory of the posterior SC nerve into that of the denervated fibres of the SC territory at 3 weeks and subsequent return of the posterior SC innervation to its own place after regeneration of the SC nerve.

reduced to that found on the control side and the contraction border had returned to its original position, as was also the case after 6 weeks.

A series of animals was examined in the same way at intervals of 1–7 weeks after cutting the SC nerve. Three axolotls were allowed to survive for 1, 2 or 3 weeks, three survived 5 weeks and one 7 weeks after operation. Figure 7 shows examples of the results of mapping the SC muscle at different intervals after cutting the main nerve. As in the crush experiments, little change in the size of the posterior SC nerve field was found during the first 2 weeks. A considerable increase in size was found after 3 weeks, with long latency (average 3.5 ms) large and small junction potentials characterizing the edge of the expanded region. There was no evidence either from observing contraction or from microelectrode recordings that the SC nerve had begun to reinnervate its own muscle fibres.

Five weeks after cutting the SC nerve, the posterior SC nerve had spread even further across denervated muscle fibres and effective connections were found in anterior muscle fibres. Action potentials were recorded close to its own territory, but most responses in the newly-innervated area consisted of long latency (average 6.1 ms) large junction potentials and

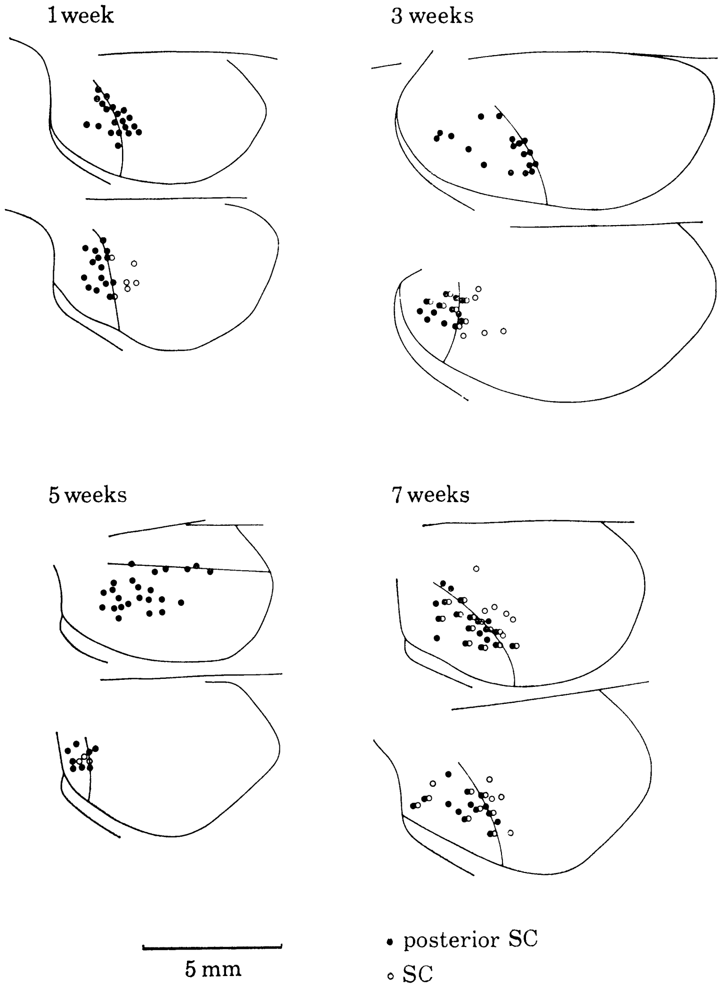

FIGURE 7. As in figure 6 except the SC nerve was cut not crushed. The longer period of denervation of fibres previously innervated by the SC nerve allows time for greater invasion of posterior SC innervation, into the SC nerve territory. Even so upon regeneration of the SC nerve (7 weeks) the posterior SC innervation becomes reconfined to the postero-lateral corner of the muscle.

smaller junction potentials in the region of the border. There was no sign in this muscle of SC nerve regeneration, probably because a large section of nerve was removed during that operation. In one of the 3 axolotls in this group there was no expansion at all. Electron microscopic examination of the supposedly denervated region of this muscle showed many immature nerve endings (we thank L. R. Marotte for this). The expansion may therefore have been prevented by collateral innervation from some other unidentified source, or the SC nerve may have regenerated but been damaged during the dissection prior to the electrophysiological experiment.

At 7 weeks, the SC nerve had regenerated, functionally reinnervated its own territory, and

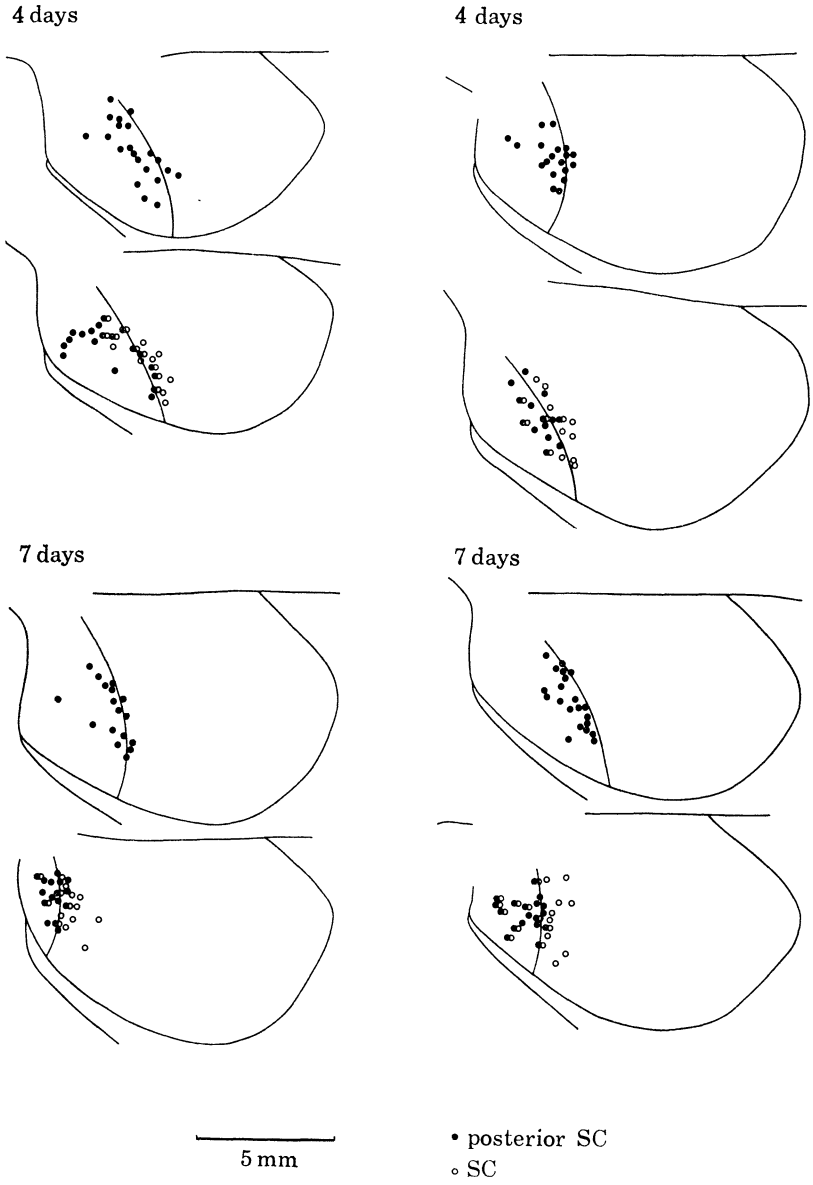

Figure 8. Symbols as in figures 6 and 7. Effect of recutting the SC nerve after it had previously been cut, to allow spread and retraction of the posterior SC nerve innervation (see text for further details). Note that after 4 days there is no significant re-expansion of the posterior SC territory. After 7 days there is considerable expansion, more than would be expected when the nerve was cut the first time.

had begun to overlap the territory of the posterior SC nerve. The smaller nerve was found to be effective only in the posterior corner of the muscle. Some small junction potentials were evoked in anterior fibres. The border of the posterior SC nerve field was reestablished in its original position.

In four animals, the SC nerve was cut a second time and the muscles mapped at 4 and 7 days after the 2nd operation. Sufficient time was allowed between the two operations for complete regeneration of the SC nerve to have occurred. (7 weeks, 7½ weeks, 5½ months and 6 months respectively). The changes in the innervation of the muscle after recutting the SC nerve are shown in figure 8.

At 4 days there was virtually no change in innervation territories. In both preparations, the contraction border and associated points at which junctional activity could be recorded after stimulation of the posterior SC nerve in the operated muscle coincided with that of the opposite control. After 7 days there was a definite increase in the area of innervation of the SC nerve. Action potentials and large and small junction potentials were found in the foreign territory and close to the border, but no long latency activity characteristic of the initial spread of innervation was recorded.

4. Discussion

(*a*) *Normal innervation*

From a developmental point of view the supracoracoideus muscle is interesting in that it has a highly stable pattern of innervation whereby control of the muscle is shared between two peripheral nerves. Differences between animals occur only in the amount of the muscle that is innervated by the posterior SC nerve, but even so there is good bilateral symmetry. There is no anatomical dividing line in the muscle, such as an intramuscular connective tissue septum to help maintain the unequal innervation of the two nerves. With cholinesterase staining there is no obvious difference in the nature of innervation of the two nerve territories and no anatomical discontinuity of innervation is apparent at the boundary. The electrophysiology of the border region shows that the synaptic efficacy of the posterior SC nerve does not decline abruptly but that there is a region in which subthreshold junctional potentials from this nerve can be elicited. Similar small junction potentials occur on stimulating the SC nerve and recording at the posterior margin of its territory. Each fibre from which a subthreshold small junction potential may be recorded gives evidence of a highly effective junctional potential from the other nerve. Small junction potentials from both sources of innervation are never found together in recordings from one muscle fibre. The converse does not hold. Right on the border there are shared muscle fibres that show effective synaptic transmission from both nerves.

One is led to think that the developmental mechanism that maintains the territories does not stop the formation of synaptic connections by either nerve in the territory of the other, but that for each muscle fibre the innervation from one source tends to dominate by reducing the efficacy of convergent terminals from the other source. Occasionally a balance is struck, but in general fibres anterior to the border do not tolerate posterior SC nerve terminals when connections are present from the SC nerve. Similarly, towards the posterior border of the SC nerve territory, terminals from this source are set at a disadvantage to those from the posterior SC nerve. Since junction potentials are recorded, but no muscle contraction results, these are naturally occurring functionless synapses. The question then is, how are they prevented from producing sufficient postsynaptic depolarization to be effective? They may produce or release very little transmitter, or the postsynaptic membrane may not respond adequately. A presynaptic deficit could arise if the terminals were very small because their growth was inhibited, if they transmitted impulses badly or stored only a few quanta (Dennis & Miledi 1974*a*, *b*; Dennis 1975). It could also arise via a physiological control of a transmitter release mechanism that was sensitive to developmental messages. A postsynaptic deficit could result from a reduced number of receptor sites, or a defective linkage between the cholinoceptive mechanism and that controlling ion permeability (Tonge 1974). These are methods for finding out which one or which combination of these mechanisms is involved.

(*b*) *Denervation and reinnervation*

About 2 weeks after cutting the SC nerve the area of muscle controlled by the posterior SC nerve begins to expand. Small junction potentials are less often seen at the old border region, and the new border shifts into the old SC territory. Junction potentials at the advancing edge of innervation are often small and do not facilitate as much as normal with repetitive stimulation. They differ from the small border potentials in that their latency from nerve stimulation is 2 or 3 times longer than normal. Because we did not record the action potentials in the nerve we cannot make reliable estimates of conduction velocity but it seems reasonable that longer latency reflects slower conduction in thin axonal sprouts. Provided the SC nerve does not regenerate, the posterior SC nerve territory spreads slowly over the muscle, taking more than 5 weeks to approach the anterior border. We do not know whether the rate of collateral innervation is constant or whether it slows as it advances into foreign musculature. The new endings mature normally and become electrophysiologically indistinguishable from those in the home territory of the nerve.

When the SC nerve regrows to reinnervate its own area of the muscle, which may be within 2 weeks of the nerve being crushed, or more than 5 weeks after removing a section of nerve, it begins at the anterior edge of the muscle and moves across to reach its normal limit near the posterior border. In the area, previously cross-innervated by the posterior SC nerve, no effective foreign terminals remain, although occasional small junction potentials are seen. In what must have been the original territory of the posterior SC nerve, there are shared muscle fibres with the balance tipping towards the posterior SC nerve towards the posterior border. The pattern of innervation that is restored is extremely precise – to within a few muscle fibres of the position of the border on the opposite side and is electrophysiologically identical with a natural one.

To see whether the previously effective collateral innervation was permanently or temporarily incapacitated we did 4 experiments in which the SC nerve was cut twice. Once to allow collateral sprouting and regrowth, and a second time to remove the competing influence of the SC nerve in its home territory. Four days after cutting the nerve the second time the posterior SC nerve territory was of normal size. By one week it was decidedly larger than normal and of a size that takes some 3–4 weeks to produce by collateral sprouting. There were no long-latency junction potentials. Our conclusion is that the repression of foreign collateral innervation is reversible, given removal of competition from the correct nerve. Repression of foreign connections on muscle fibres normally controlled by only one nerve may simply be a more extreme version of the competitive mechanism that forms a border between innervation territories by partial and then complete block of synaptic depolarization from terminals that stray too far into a foreign nerve territory.

These experiments are all difficult to do, more so the latter ones, because one cannot follow the full cycle of degeneration and regeneration except by sampling different animals at different times, and one must rely upon the opposite, unoperated muscle for delineating the territories on the operated side. Nevertheless, they suggest that in this situation there are two mechanisms of extending innervation; collateral sprouting and derepression of partially or perhaps completely ineffective junctions. The restoration of the correct pattern after nerve regeneration almost certainly involves competitive repression of synaptic transmission, a reversible process – at least to begin with.

There seems enough evidence now to say that after regeneration of cut nerves selective reconnection of motoneurones with the muscles they previously innervated, however it is done, is very largely and perhaps completely responsible for the return of coordinated muscular movement. It remains to be seen whether similar mechanisms govern normal neuromuscular development and whether the same principles might apply to the formation of interneuronal connections in the vertebrate central nervous system.

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Discussion

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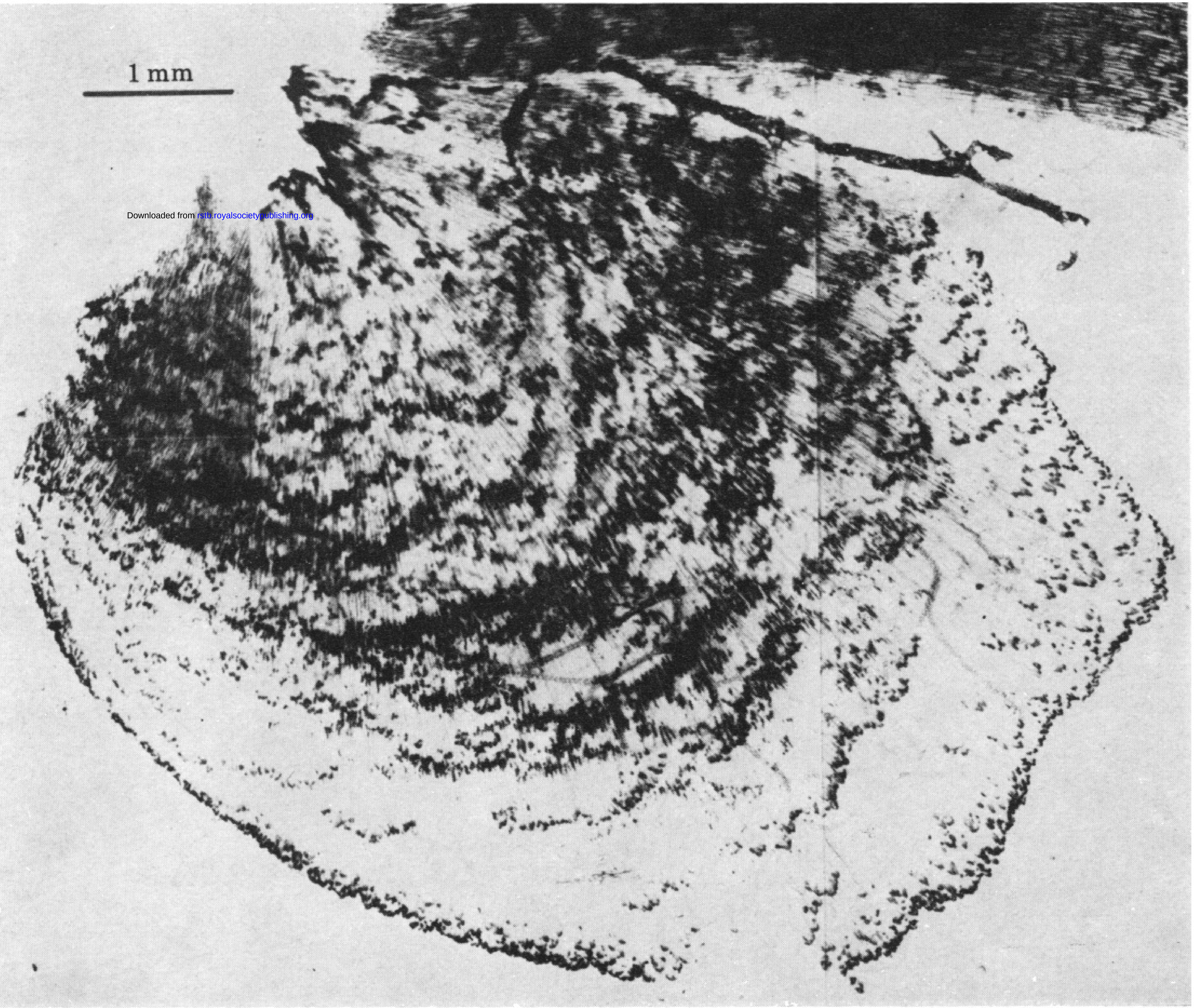
The possible existence of 'silent', 'non-functional' or 'repressed' synapses is a difficult one to assess from the kind of evidence which Dr Mark presents. His original interpretation was based on experiments on the eye-muscles of goldfish (Mark, Marotte & Mart 1972). However, this work has now been convincingly refuted by the experiments of Scott (1975), who also has new results which I am able to communicate here; the behavioural findings of Mark *et al.* are explainable by regeneration of the muscles which had been removed as part of the operative procedures. Dr Mark has now suggested that the existence of two sets of muscle fibres (large and small) which supply the eye muscle are responsible for the disparity. Dr Scott has consequently repeated her detailed electrophysiological analysis with particular attention to the type of muscle fibre investigated, and has shown that 'repression' of foreign junctions does not occur on either type of muscle fibre. When we come to the urodele a different situation prevails. Dr M. Dennis (personal communications) has recorded intracellularly from muscle fibres in the adult newt and has shown that under conditions of 'competition' between a foreign and an appropriate nerve, re-establishment of correct innervation can cause some kind of 'repression' of the incorrect synaptic input, whose terminal transmission gradually declines in quantal content. However, more work is needed before the existence of a permanent repression of synapses is established.

References

Mark, R. F., Marotte, L. R. & Mart, P. E. 1972 The mechanism of selective reinnervation of fish eye muscles IV. Identification of repressed synapses. *Brain Res.* **46**, 149–157.
Scott, S. A. 1975 Persistence of foreign innervation on reinnervated goldfish extraocular muscles. *Science, N.Y.* **189**, 644–646.



IGURE 2. A photomontage of a section of the whole of the supracoracoideus muscle stained for cholinesterase to show nerve endings. Note how they are arranged in arcs across the line of the muscle fibres.