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MOTOR INNERVATION WITHIN SUPERNUMERARY LEGS OF COCKROACHES

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SUMMARY

1. Clusters of legs having prothoracic and metathoracic origins were grown from the metathoracic coxa of the cockroach.
2. Of these legs, those which were innervated contained only one, or occasionally two, of the three major nerves innervating the cockroach leg.
3. Stimulation of a particular leg nerve (no. 3, 5 or 6) evoked movement at the same joints and in the same directions in a leg having only one nerve as in a normal leg.
4. Stimulation of a particular metathoracic nerve generally produced the same movements in a prothoracic leg transplanted to the metathoracic site as it did in a regenerated or intact metathoracic leg.

INTRODUCTION

In the preceding paper (Westin & Camhi, 1975), we showed that metathoracic nerves of the cockroach, regenerating towards clusters of legs having different segmental origins, enter prothoracic legs as readily as metathoracic legs. A remaining question is: when a single metathoracic nerve enters a metathoracic leg, does it retain its specificity for certain muscles, or does it spread out to synapse with muscles normally innervated by other nerves? Also, does a single metathoracic nerve which enters a prothoracic leg synapse preferentially with muscles homologous to those it normally innervates? Answering these questions would help to understand any signalling system by which selective neuromuscular contacts become specified.

In amphibians and teleost fish, nerves can be forced to innervate inappropriate muscles, but when allowed to regenerate without interference, they apparently establish normal neuromuscular contacts (Grimm, 1971; Marotte & Mark, 1970*a*, *b*; Mark & Marotte, 1972; Mark, Marotte & Mart, 1972; Cass, Sutton & Mark, 1973). In cockroaches, individually identified leg motor neurones re-innervate selectively their correct muscles following axotomy (Young, 1972; Pearson & Bradley, 1972). In neither case, however, is it known whether neurones normally innervating all the muscles of the leg must be present in order to prevent the formation of inappropriate synapses. Early experiments on amphibians suggested that a single nerve might

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spread to vacant muscles normally innervated by other nerves (Weiss, 1937*a*, *b*), but other interpretations of this experiment now seem more plausible (Grimm, 1971; Cass *et al.* 1973).

In amphibians, forelimb nerves can be made to innervate hindlimbs and *vice versa* (Székely, 1963; Hughes, 1964). The co-ordinated movements produced by these abnormally innervated legs suggest that the neurones can locate, in foreign limbs, muscles homologous to those they normally innervate; but the question has not been answered definitively.

Our experimental situation offers two major advantages over that in amphibians for answering questions about neuromuscular specificity. First, a leg grown as one of a cluster is usually innervated by only one nerve (Westin & Camhi, 1975). Secondly, none of the three major nerves of a cockroach leg contains motor neurones for all of the muscles in the leg. Thus, it is possible to produce legs containing nerves which normally innervate only a fraction of their muscles.

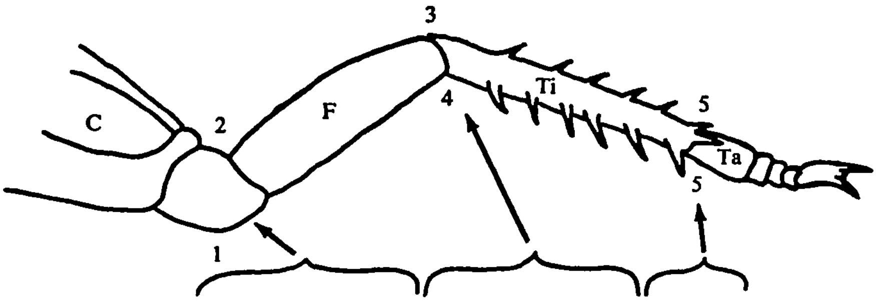
In this paper we show that electrically stimulating a particular nerve evokes the same joint movements in legs innervated solely by this nerve as in legs which are innervated normally. Moreover, the same applies if the leg in question is a prothoracic leg grown as a member of a cluster at the metathoracic site.

MATERIALS AND METHODS

In this study, we used cockroaches (*Periplaneta americana*) with normal legs, singly regenerated legs, and clusters of regenerated legs. The methods for growing the latter types have already been described (Westin & Camhi, 1975). Some of the legs in the clusters were of prothoracic origin, others were metathoracic. All animals were tested within 1 or 2 days of the appearance of a single regenerated leg or a cluster of legs. Having determined which leg of a cluster a given nerve had entered (Westin & Camhi, 1975), we then observed in detail the movement evoked in the leg upon stimulation of the nerve. For comparison, we also observed movements produced by stimulating the same nerve in normal and in singly regenerated metathoracic legs. (Attempts to record electrical activity of the muscles were abandoned because of the fragility of the legs in clusters, and the disorganized state of their muscles.)

For stimulation, the nerve to be tested was transected near the ganglion (proximal to all branches) and a suction electrode was placed over the distal end. The stimulator (Grass SD 5) delivered pulses of 1–10 msec duration, 0·08 to 8 V, as single shocks or trains at a frequency of 10 Hz, with the inside of the electrode negative. Beginning at low voltage and duration, we increased these parameters until we observed movement of the leg, and then continued to increase the stimulus until it was about ten times this threshold, or until it reached 8 V.

During stimulation the legs were viewed through a stereomicroscope, so that movements were readily visualized. Records were kept on the incidence of flexion and extension at each joint, the particular nerve stimulated (metathoracic 3, 5 or 6), and the category of leg (normal, singly regenerated, prothoracic in cluster, metathoracic in cluster). If a leg moved at more than one joint, the segments were held one at a time with forceps in order to determine whether each movement was active. Only movements distal to the coxa were considered.



Nerve stimulated	Type of leg	1 Ext C-F	2 Flex C-F	3 Ext F-Ti	4 Flex F-Ti	5 Flex/Ext Ti-Ta.	number of nerves
N3	Normal	11	13	18			27
	Single	4	3	2			19
	Cluster	4	1	1			51
N5	Normal	25	3		20	6	31
	Single	4	1		8		16
	Cluster	10	7		15	5	51
N6	Normal	12	8				21
	Single	5					16
	Cluster	13	6				51

Fig. 1. Movements obtained in different types of legs in response to stimulation of the three major leg nerves. Nerves: N3 = nerve 3, N5 = nerve 5, N6 = nerve 6. Legs: Normal = normal intact control legs, single = singly regenerated legs, cluster = legs which regenerated as members of a cluster. Movements: Ext = extension, Flex = flexion, Flex/Ext = either flexion or extension, C-F = coxo-femoral joint (includes movements at coxo-trochanteral joint and those at trochantero-femoral joint), F-Ti = femoro-tibial joint, Ti-Ta = tibio-tarsal joint. Number of nerves = total number of each type of nerve stimulated.

RESULTS

This study is based on 283 stimulated nerves, of which 79 were from normal control animals, 51 from those with singly regenerated legs, and 153 from those with clusters of legs. Nearly all (96%) of the nerves to normal legs evoked detectable movements, while only 45% of those to singly regenerated legs, and 33% of those to clusters did so. Of 49 legs in clusters which showed detectable movements upon stimulation of their nerves, 75% contained only one detectable nerve.*

Normal legs, singly regenerated legs, and legs in clusters generally responded

* Of these 49 innervated legs, 37 apparently contained only one nerve, eleven contained two nerves, and one contained three nerves.

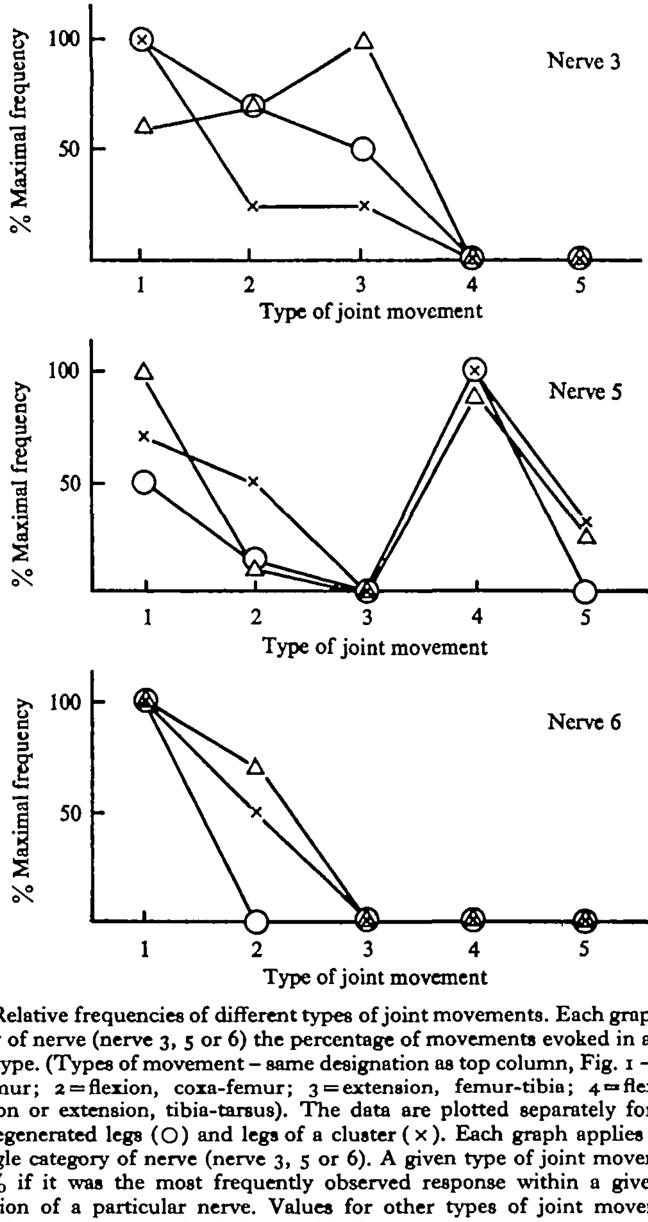
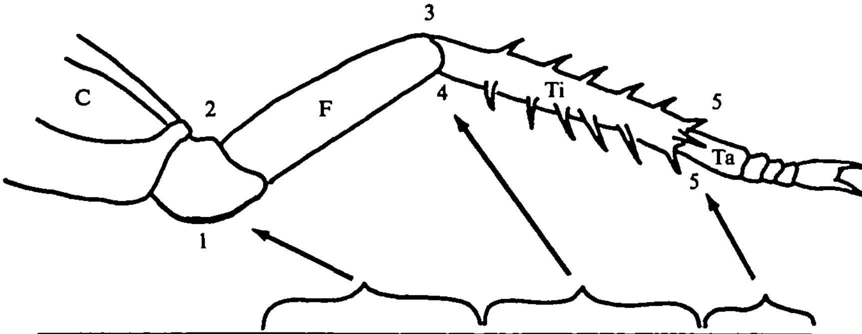


Fig. 2. Relative frequencies of different types of joint movements. Each graph plots for a single category of nerve (nerve 3, 5 or 6) the percentage of movements evoked in a leg which were of a given type. (Types of movement – same designation as top column, Fig. 1 – are: 1 = extension, coxa-femur; 2 = flexion, coxa-femur; 3 = extension, femur-tibia; 4 = flexion, femur-tibia; 5 = flexion or extension, tibia-tarsus). The data are plotted separately for normal legs (△), single regenerated legs (○) and legs of a cluster (×). Each graph applies to the stimulation of a single category of nerve (nerve 3, 5 or 6). A given type of joint movement is designated as 100 % if it was the most frequently observed response within a given class of legs to stimulation of a particular nerve. Values for other types of joint movement in the same class of legs are computed relative to this figure.

with movements at the same joints when a given nerve was stimulated (Fig. 1).* Moreover, there is some specificity at individual joints, since nerve 3 produced only extension at the femoro-tibial joint in all three types of leg, and nerve 5 produced only flexion. For other combinations of nerve and joint, movements occurred in different directions in different animals (e.g. at the coxo-femoral joint, nerve 3 evoked flexion in some ainmals and extension in others).

* One exception to this statement is that nerve 5 never evoked any tarsal movements in singly regenerated legs, whereas it did in normal legs and those in clusters. Also nerve 6 never evoked flexion of the coxo-femoral joint in singly regenerated legs, whereas it did in both other types of leg.



Nerve stimulated	Type of leg	1 Ext C-F	2 Flex C-F	3 Ext F-Ti	4 Flex F-Ti	5 Flex/Ext Ti-Ta
N3	Pro	3				
	Meta	1				
N5	Pro	4	3		12	5
	Meta	3	3		2	
N6	Pro	8				
	Meta	5	4			

Fig. 3. Types of joint movement evoked in legs of different segmental origins in response to stimulation of the only nerve entering the leg. Types of joint movement and nerves – same as for Fig. 1. Types of leg: Pro, leg of prothoracic origin; Meta, leg of metathoracic origin.

There was some variability, even in normal animals, in the responses to nerve stimulation, some movements occurring much more commonly than others (Fig. 1). These differences may reflect variability between preparations in the thresholds of specific motor axons, or damage to different axons in the course of stimulation. Nevertheless, in different categories of legs, there are striking parallels in the responses to nerve stimulation. These are revealed most clearly by plotting the relative frequencies of movements at the different joints, evoked in the different types of leg (Fig. 2). (The lack of correspondence for nerve 3 may result from the very low sample size – Fig. 1.)

The types of joint movement evoked in legs of clusters (most of which contained. only one nerve) were generally independent of the segmental origin of the leg (Fig. 3). Each movement produced at least once in a prothoracic leg or a metathoracic leg was also produced at least once in the other, with two exceptions.* The pattern of innervation is very much like that in normal legs (Fig. 1).

The total number of different joint movements evoked in the legs of a cluster, upon stimulation of the three major nerves (3, 5 and 6), did not vary significantly

* Only prothoracic legs showed movements at the tibio-tarsal joint upon stimulation of nerve 5, and only metathoracic legs showed flexion at the coxo-femoral joint upon stimulation of nerve 6. These exceptions are probably not significant due to the small numbers of animals involved.

with time or with number of moults between the transplanting operation and the emergence of a cluster. Movements at more distal joints also failed to increase with time or with number of moults. Thus, the relatively small number of movements which could be evoked in clusters is apparently not due to inadequate time for nerve regeneration. In fact, the time required for emergence of clusters was, on the average, much longer than that for singly regenerated legs, from which a higher frequency of responses was obtained.

DISCUSSION

We have shown that in cockroach legs which are innervated by one nerve rather than the normal complement of three, stimulation of this nerve evokes movement of the same joints, and generally in the same directions, as it does in a normal leg. This suggests that the nerve does not make functional contacts with inappropriate muscles, even though these are deprived of their normal innervation. As a specific example, nerve 6 does not form functional excitatory synapses which result in visible contraction of muscles beyond the coxo-femoral joint (Figs. 1 and 2). Also, nerve 5 which does not evoke visible extension at the femoro-tibial joint, may grow past these extensor muscles without making synaptic contacts on them. (It is of course possible that nerve 5 does form synapses on tibial extensor muscles but that these do not produce visible movements owing to failure of the junctions or contractile mechanism, or because the contraction of the tibial flexor is always stronger.)

Nerve regeneration is accompanied by complete functional recovery in lower vertebrates, but not in mammals. It has been proposed that this is due to the fact that in the former, muscle fibres can be polyneuronally and multiterminally innervated, whereas mammalian muscle fibres normally receive only one motor neurone (Mark, 1965; Mark, von Campenhausen & Lischinsky, 1966; Cass *et al.* 1973). In lower vertebrates it has been shown that motor neurones can innervate inappropriate muscles, and that these improper contacts can be displaced later, if the proper motor neurone makes contact (Marotte & Mark, 1970*a*, *b*; Mark & Marotte, 1972; Mark *et al.* 1972). If selective reinnervation is dependent on this mechanism, then some incorrect synapses might be expected when a limb is partially innervated. Insect muscle fibres are also polyneuronally innervated, but functionally proper re-innervation, at least to the extent demonstrated in this study, is not dependent on the presence of a complete set of neurones.

We have also shown that metathoracic nerves generally produce the same types of joint movement in prothoracic legs as they do in metathoracic legs. This is in accord with previous work showing that identified mesothoracic neurones synapse with homologous muscles in transplanted metathoracic legs (Young, 1972).

The observation that an individual nerve produces the same movements of a leg whether or not other nerves are present, and regardless of the segmental origin of the leg, is most simply explained by selective innervation of the muscles appropriate to that nerve. Other possible interpretations are that the nerve has induced only its own muscles to develop, or that it has allowed only its own muscles, once developed, to survive.

Histological sections of the leg clusters revealed muscles which were more sparse and disorganized than in a normal leg. Since they could not be individually identified, it was impossible to determine whether only those muscles normally associated with a given nerve were present.

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