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SPECIFIC RE-INNERVATION OF LIMBS TRANSPLANTED BETWEEN SEGMENTS IN THE COCKROACH, *PERIPLANETA AMERICANA*

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INTRODUCTION

The specificity of neuronal connexions and their capacity for regeneration are subjects of increasing interest. The valuable work on Amphibia has been reviewed by Gaze (1970). In the present report advantage has been taken of the suitability of insects for this type of work. Insects will accept the same range of experiments as the amphibia but allow them to be carried to a new level of accuracy. This is because single nerve cells can be identified individually in the insects, which is not possible as yet in the vertebrates.

The neuromuscular connexions of the limbs of the cockroach, *Periplaneta americana*, have been used here. These are now well studied and the nerves and muscles have been described and numbered. Details of these notations, which are used here, will be found in the recent compilation of Guthrie & Tindall (1968). More recently the physiology of the motor supply has been elucidated by Pearson & Iles (1970, 1971) and Iles & Pearson (1971). It is known that the motor neurons of the cockroach can regenerate and restore functional contact with the muscles (Bodenstein, 1957; Guthrie, 1962, 1967; Jacklet & Cohen, 1967).

Following the introduction of the dye procion yellow by Stretton & Kravitz (1968) it has become possible to mark individual neurons which have been penetrated with a microelectrode. In the locust Bentley (1970) and Hoyle & Burrows (1970) have shown that the motor neurons can be triggered by intracellular stimulation of the cell body, which is then marked by electrophoretically injecting the cell body with procion yellow from the microelectrode. This makes it possible to locate the cell bodies of motor neurons supplying particular muscles.

In order to carry out meaningful experiments on the specificity of the connexions of motor neurons to their muscles it is necessary to identify individual motor neurons independently of their demonstrated connexions to given muscles. In the cockroach I have been able to recognize individually the larger motor neuron cell bodies on the basis of their size, shape and position and so to allocate a number to each of them. In this way numbered cell maps can be produced and have been published by Young (1969) for the mesothoracic ganglion and by Cohen & Jacklet (1967) who employed the same notation for the metathoracic ganglion.

In an earlier paper (Young, 1969), I drew attention to the close similarity between the serially homologous cell bodies in the mesothoracic and metathoracic ganglia of

the cockroach. This naturally leads to the question of whether these serially homologous neurons in the two segments supply serially homologous muscles. If they do, a further question is whether, upon transplanting a limb from one segment to another, an identified motor neuron will regenerate to that transplanted muscle which is the serial homologue of its own.

This paper attempts to answer these questions at the level of single cells using the identified motor neurons of the cockroach, whose connexions have been demonstrated in normal and regenerate animals by means of intracellular stimulation and marking with procion yellow.

METHODS

The physiological preparation

Animals were anaesthetized with carbon dioxide, decapitated and pinned out, ventral side uppermost. The animals were pinned in a relaxed position and not stretched out at all. Movements of the animals were reduced to a minimum by sealing the legs to the body with insect wax. The limb under study was sealed to the thorax only at the coxa, leaving all the segments distal to the coxa completely free to move. A flap of cuticle was removed to expose the ganglion, which was then supported from underneath by a plastic ring, 1 mm in diameter, held in position by a micromanipulator. These preparations held the ganglion sufficiently steady for stable penetration of cell bodies with a microelectrode.

The superficial tracheal supply was removed and the ganglion was de-sheathed over the neurons being studied. The ganglion was kept flooded with saline, made up according to the formula of Yamasaki & Narahashi (1959). Petroleum jelly was used to prevent the saline from draining away over the sides of the animal. The muscles under study were exposed by dissection, so that they could be watched during stimulation, and were kept moist with the same saline solution. A flow of carbon dioxide was maintained over the animal during preparatory work but the animal was allowed to recover completely (10–15 min) before the experiment began. It was found that motor neurons could not be fired by intracellular stimulation of the cell body while the animal was anaesthetized with carbon dioxide.

Stimulation and marking of cell bodies

Motor neuron cell bodies were rendered visible under a dissecting microscope by illuminating the ganglion with a light guide at an oblique angle. This was held in a micromanipulator so that the tip of the light guide could be moved in all directions over the surface of the ganglion to achieve the best lighting effect.

Glass microelectrodes were filled with a 4% solution of the dye procion yellow. They were connected to the input of a Grass P17 pre-amplifier via a switch which allowed the electrode to be switched either to the pre-amplifier or to a Tektronix 160 series stimulator. For this work the electrode resistance was not important, and the tip of the electrode was usually broken off by gently advancing the electrode against the light guide before penetrating cell bodies. This made stimulation and marking of cell bodies more consistently successful. The electrode was assumed to have penetrated the cell body when a stable resting potential was observed. This usually fell between 15 and 40 mV but was sometimes less, rarely more. The electrode was

then switched to the stimulating position and positive current was passed to fire the cell; 100–300 nA was usually sufficient. The muscle which the cell body supplied was identified by directly observing a twitch in the exposed muscle (only fast motor neurons were studied) and, where appropriate, a movement of a segment of a limb. Once this had been observed unequivocally, the cell body was marked by passing a negative current of 50 nA until the cell body became visibly coloured with dye under the microscope. To facilitate the change-over the stimulus pulses were the same for both stimulating and marking the cell body, having a pulse duration of 100 ms and a pulse interval of 320 ms. More than one cell could be marked per ganglion without loss of identity. Some fifty successful preparations form the basis of this paper.

Histological procedures

At the end of an experiment the ganglion was fixed in alcoholic Bouin's fluid (Pantin, 1946). The ganglion was first flooded with fixative to help to preserve its shape and then dissected out and placed in fixative for 2 h or more. Long periods of fixation did not affect the ganglion adversely. The ganglia were dehydrated and embedded in paraffin wax. The blocks were carefully orientated under a dissecting microscope so as to cut the ganglia as nearly as possible transversely. Sections were cut at 10 μm.

Unstained sections were cleared in xylene and mounted in a non-fluorescent medium (Permount) and examined under a Zeiss Photomicroscope II set up for fluorescence microscopy. With procion yellow, Zeiss excitation filter no. BG3 and barrier filter no. 53 gave the best results. The injected cell bodies were located under the microscope and notes were made of the particular sections in which each was to be found.

After this was completed, the coverslips were dissolved off the slides with xylene and the sections were re-treated as for a normal histological preparation. They were stained with pyronine–malachite green (Baker & Williams, 1965) and mounted in canada balsam for long-term storage. Used in this way, pyronine–malachite green does not retain its histochemical specificity but is merely a convenient stain which stains cell bodies and leaves the neuropile unstained. These sections could then be studied at length and the marked cell bodies could be identified by reference to a cell map. Only occasionally was the distortion introduced by de-sheathing so severe as to prevent reliable identification of cell bodies. Favourable preparations were used to produce additional cell maps using the reconstruction technique of Pusey (1939) with the rapid modification of Potts (1966).

The limb transplant operations

Nymphal cockroaches, which were two to four moults from the imaginal moult, were used for transplant operations. The host animal was selected at moult by its white cuticle and operated on 1–3 days later. In this way it had a complete inter-moult period in which to incorporate the transplanted limb. The transplanted limb was taken from another animal of the same size. The host was lightly anaesthetized with carbon dioxide and carefully secured in a dish with Plasticine. The entire left-hand mesothoracic limb of the host was severed at its joint with the thorax and removed. Immediately, the left-hand metathoracic limb of the donor was severed

similarly and transplanted into the place of the host's left-hand mesothoracic limb. It was sealed in place with insect wax. Aseptic precautions were found to be unnecessary. Fifty-three such operations were carried out.

Each host animal with its transplanted limb was isolated in a numbered plastic luncheon box, with a large gauze window in the lid. Each animal was supplied with food and water and a record was kept of its subsequent development. A minimum of 5 months was allowed to elapse before these animals were experimented upon in any way.

The more successful transplants were studied during normal walking movements which were recorded on cine film. Each subject was placed in a chamber similar to that described by Delcomyn (1971*a*) and was filmed from 2·5 ft with a Bolex H16 camera at 64 frames/s. Ilford Pan F negative film was used and suitable sequences were selected for printing of individual frames for detailed study.

RESULTS

The identity of the motor neurons

The mesothoracic motor neurons form the central reference point of this study, the purpose of which is to examine the specificity of connexions between these identified motor neurons and identified limb muscles. For a given motor neuron to be a useful subject for this kind of study it should have the following properties. First, it is important that its cell body should have a distinctive anatomical appearance and location within the ganglion so that it may be recognized with certainty from histological sections and cell maps. Second, it is desirable that the neuron should have important and easily recognizable connexions to one or more muscles. For this reason only fast neurons were studied because their action is easy to observe in the normal animal and so provides a clear-cut test of the presence or absence of successful regeneration. A third desirable feature is the arbitrary characteristic that the cell body should be relatively easy to penetrate and stimulate with a microelectrode; the motor neurons vary consistently in this quality without any obvious reason.

Three mesothoracic motor neurons have been specially chosen which fulfil these conditions. All three are concerned with fast depression (= extension) of the leg. The most outstanding of these is cell body no. 30, which is one of the largest and most conspicuous in the whole ganglion. Its largest dimension is about 65 μm (in fixed material) and it is situated in a prominent position toward the posterior end of the main anterior group of cell bodies (Plate 1*a*). In transverse sections cell body no. 30 has a tilted appearance because the axon leaves at about 45° to the vertical (Plate 1*b*). These features remain evident in de-sheathed experimental ganglia after the cell body has been filled with procion yellow (Plate 1*d*). Intracellular stimulation of this cell body produces a vigorous twitch in the coxal depressor muscles (135*d'*, *e'*, 136, 137) and a concomitant rapid extension of the femur. This clearly corresponds with the neuron described by Pearson & Iles (1971) as the only fast neuron to the coxal depressor muscles, which they term D_f.

The metathoracic homologue of mesothoracic cell no. 30 is metathoracic cell body no. 28, as was shown in Young (1969, see figs. 6–9) on purely morphological criteria. Intracellular stimulation of metathoracic cell body no. 28 produces a vigorous

twitch in the coxal depressor muscles (177*d'*, *e'*, 178, 179) and a powerful extension of the femur. This cell is thus the metathoracic D_f. This result accords with a recent study by Iles (1972) in which the metathoracic D_f was provisionally identified with cell body no. 28 using a quite different method of getting procion into the cell (Iles & Mulloney, 1971).

The second mesothoracic cell chosen here is motor neuron no. 29, which is always found closely alongside cell body no. 30. It is somewhat smaller than cell body no. 30, its greatest dimension being 50–55 μm (in fixed material), and it is situated nearer the mid line (Plate 1*a*). Out of many hundreds, I have only seen one ganglion in which this relative positioning was reversed and no. 30 was situated nearer the mid line. Cell body no. 29 also has a tilted appearance in transverse section because its axon leaves on a slanting course (Plate 1*b*). These characteristics are again clearly evident when the cell body has been marked with procion yellow in experimental ganglia (Plate 1*c*). Intracellular stimulation of this cell body produces a twitch in the first tergal muscle of the trochantin (118) and the sternal promotor of the coxa (126), which are adjacent muscles acting on the coxal rim, effectually in series with the coxal depressor muscles.

The metathoracic homologue of mesothoracic cell body no. 29 is metathoracic cell body no. 27, again as shown in Young (1969, figs. 6–9). Intracellular stimulation of this cell body produces a twitch in the first tergal muscle of the trochantin (161) and the first episternal promotor of the coxa (167). While the arrangement is not identical with the mesothoracic segment, these muscles are clearly the serial homologues of 118 and 126.

The third example is mesothoracic cell body no. 18, which is a rounded cell body about 50 μm in diameter (in fixed material) without any obvious axon hillock. It is situated in a conspicuous position on the antero-lateral portion of the ganglion. It is readily penetrated with a microelectrode, and intracellular stimulation produces a vigorous twitch in the tergal branch of the coxal depressor of the leg (135*a*) and the concomitant powerful extension of the femur. The metathoracic homologue of this cell is metathoracic cell body no. 18. The anatomical features of the two cells are again very similar in the two ganglia. Similarly, intracellular stimulation of metathoracic cell body no. 18 produces a vigorous twitch in the tergal branch of the metathoracic coxal depressor, 177*a*, the serial homologue of 135*a*.

Thus it is evident that serially homologous cell bodies do supply serially homologous muscles in the mesothoracic and metathoracic ganglia of the cockroach, and these results are summarized in Text-fig. 1.

The fate of the transplanted limbs

The next step in this analysis was to transplant a left metathoracic limb from a donor into the place of a left mesothoracic limb of a host as described under Methods. All 53 hosts survived the operation and the transplant was held firmly in place by the insect wax.

The normal course of events was that the isolated animals moulted once every 4 to 5 weeks until they became adult. The first moult after the transplant operation was the first real test of the operation. In the unsuccessful operations the transplant either failed to take or caused the host difficulty in moulting. In the successful

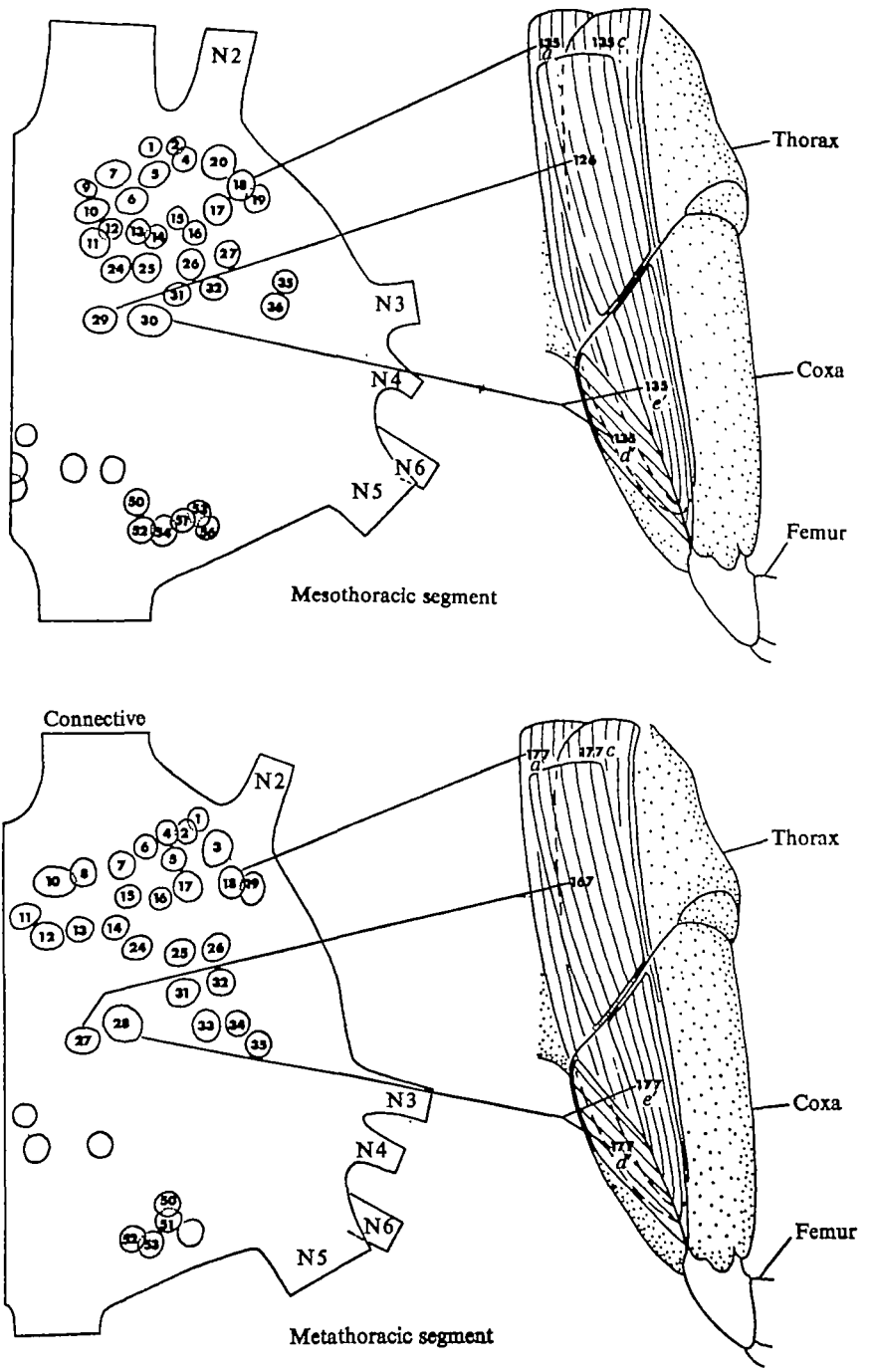

Fig. 1. Diagram illustrating the connexions of selected identified motor neurons in the mesothoracic (above) and metathoracic (below) segments. For each segment is shown: left, an accurately reconstructed half-ganglion with identified motor neurons (numbered) and peripheral nerve trunks (N2–N6), and right, a drawing of the proximal parts of the leg with numbered muscles. The connecting lines join the selected cell bodies to the muscles which they supply. Muscles 136, 137 and 178, 179 have been omitted for clarity.

operations varying amounts of the metathoracic limb became incorporated in the next instar. The minimum for a successful take was a complete coxa, which then regenerated the rest of the metathoracic leg at subsequent moults. Even where a complete metathoracic leg took at the first moult it was always small and required one or two more moults to develop to a good size.

The isolated animals often preferred to rest upside down on the gauze window in the lid of their boxes. This provided a convenient test of the functional state of the transplanted limb. At first the transplanted limbs hung down listlessly, but with time the successful transplants were held up and gripped the gauze and were protracted when the animal walked slowly across the gauze.

The final outcome of these operations after 6 months had elapsed was as follows. In 20 animals the transplant failed for one reason or another such as the animal being unable to moult successfully or the transplant withering and being replaced by a mesothoracic limb. In 18 animals the transplant took successfully but remained small, this usually being the case in animals with only one or two moults to adult. In the remaining 15 animals the transplant took successfully and, over the next three or four moults, reached a size larger than the control mesothoracic limb but never as large as the normal metathoracic limb (Plate 2).

The differences between meso- and metathoracic limbs

These transplants were undertaken in order to study whether mesothoracic motor neurons would innervate metathoracic muscles and if so which ones. For the results to be reliable it is essential that each transplant studied be positively shown to be a metathoracic limb and not merely assumed to be such from the fact of its having been transplanted. Therefore, the differences between normal mesothoracic and metathoracic limbs were described to provide a criterion by which to assess the transplant. But since the transplant takes time to 'catch up' in its development and never reaches full metathoracic size, the comparison must be extended to other than fully adult-sized limbs. Accordingly, detailed comparison was made of the meso- and metathoracic limbs of the last three instars of normal animals, and the transplants were compared with these.

Though generally very similar, the meso- and metathoracic limbs consistently differ in a number of features which do not overlap during the last three instars, and these are expressed graphically in Text-fig. 2. One of these is the relative length of the femur and tibia: in the mesothoracic limb they are very nearly equal whereas the metathoracic tibia is about 1·25 times the length of the femur (Text-fig. 2*a*). Another clear distinction involves the fine hairs that occur on the ventral surface of the femur and tibia: compared with the mesothoracic limb, these hairs are about twice as numerous on the metathoracic femur and about three times as numerous on the metathoracic tibia and the ranges of variation do not overlap (Tex-fig. 2*c*). It is rather difficult to count accurately all these hairs on a limb segment and the counts given in Text-fig. 2*c*, *d* represent the numbers visible at a single level of focus under the dissecting microscope. The limbs also differ consistently in the number and arrangement of the large spines on the femur and tibia as well as in more subtle characters such as the shape of the femur (see Plate 2).

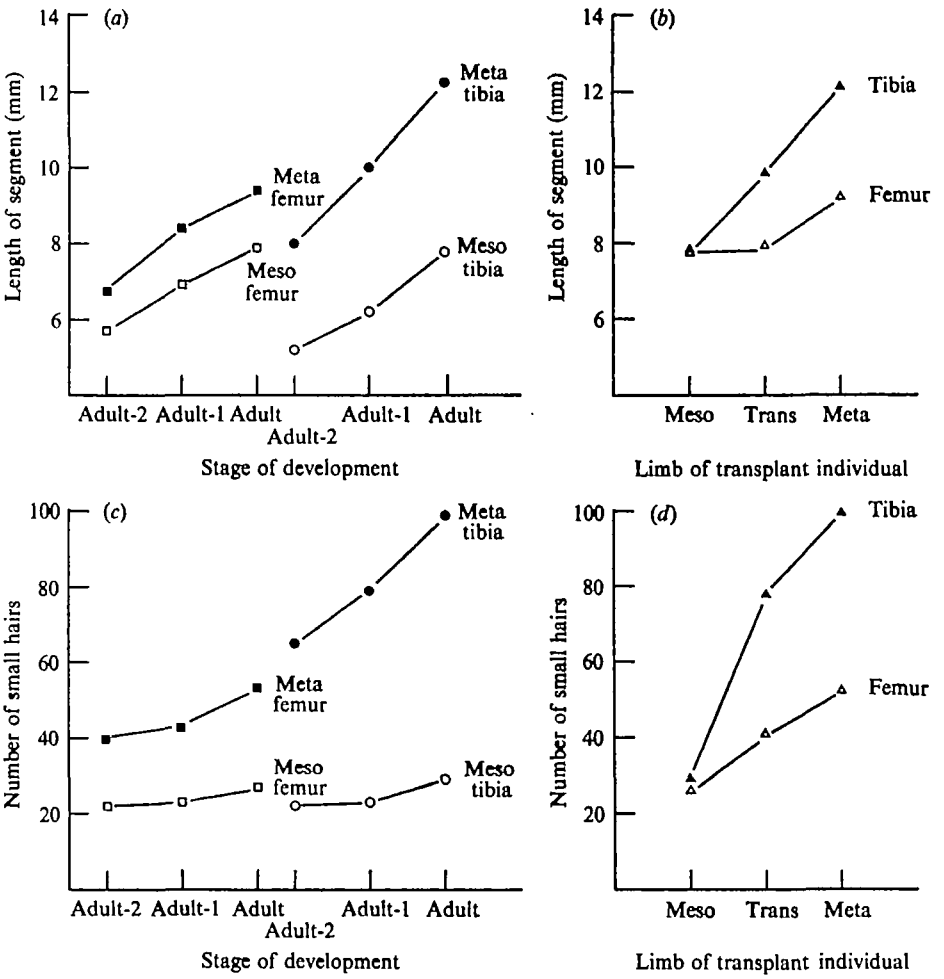

Fig. 2. Distinguishing characteristics of mesothoracic and metathoracic limbs of normal animals compared with those of animals bearing transplanted limbs. On the left (*a*, *c*) are shown the characteristics of normal limbs of animals from the ordinary culture during the last three instars (adult−2, adult−1, adult). On the right (*b*, *d*) are shown the corresponding values from transplant individuals. For these, the value is given of the normal mesothoracic limb (meso), the normal metathoracic limb (meta) and the transplanted metathoracic limb (trans). Both normal (*a*, *c*) and transplant (*b*, *d*) figures give means of samples of ten individuals.

External features of the transplants

Those features which distinguish the meso- and metathoracic limbs were examined in each of the 15 most successful transplant animals and the results are incorporated in Text-fig. 2*b*, *d*. In each of these animals details were taken for the control mesothoracic limb, the transplant and one of the normal metathoracic limbs. In the relative length of the femur and tibia the transplant clearly conforms to the metathoracic pattern (Plate 2, Text-fig. 2*b*) as it does also in the number and arrangement of large spines (Plate 2). Again the result is clear-cut when the number of fine hairs is considered, the transplant falling outside the mesothoracic range of variation and within the metathoracic range of variation (Text-fig. 2*d*, Plate 3*b*). Taking the results

presented in Fig. 2 in combination, the outcome is that the transplant corresponds closely with a metathoracic limb of the last nymphal instar. Hence there can be little doubt that in these 15 cases the transplanted limb has remained metathoracic and is not intermediate or mesothoracic in form.

Re-innervation of the transplants

The transplant operation necessarily severed the host's main leg nerve (N5), which contains the axon of mesothoracic cell 30, the D_f axon (see above), and presented the cut nerve with a new set of metathoracic muscles. In those animals where the metathoracic nature of the transplant was demonstrated, an attempt was made to penetrate cell body no. 30 and stimulate it intracellularly. Where a result was obtained, cell bodies were marked with procion yellow for identification in sections as in normal animals. In all these cases, stimulation of mesothoracic cell body No. 30 was found to produce a twitch in the transplanted metathoracic coxal depressor muscles (177*d'*, *e'*, 178, 179) with corresponding extension of the femur. Thus this mesothoracic cell had consistently re-innervated those transplanted metathoracic muscles which are homologous with its own target muscles.

A further point is that the transplant operation involves the transection of coxal depressor muscles 135*a*, *c*, long and very powerful muscles, one of which is innervated by cell body no. 18. In the transplants the host muscles 135*a*, *c* formed a butt joint with their metathoracic homologues 177*a*, *c* in the transplanted coxa. The resulting compound muscles were functional and in histological sections many fibres were seen to be continuous through the join. On the whole, the muscles in the region of the transplant were less well developed than in a normal animal and were surrounded by much wound tissue.

The host animals appeared to use the transplanted leg when walking about their boxes and, to test this more closely films were made of seven of the most successful transplants during more rapid walking in a suitable chamber. The ordinary film speeds available were not adequate for a quantitative evaluation of the kind carried out by Delcomyn (1971*a*) but they were quite adequate to evaluate a few qualitative points for comparison with the results of Hughes (1952, 1957). The films showed that in all seven animals the transplanted leg was capable of walking movements although in one of them the femuro-tibial joint was inoperative. They also showed that the transplanted leg is protracted at the correct point in the normal walking sequence R_1, L_2, R_3, L_1, R_2, L_3 as if it were the normal left mesothoracic limb (Plate 3*a*).

No systematic observations were made on the ingrowth of sensory neurons from the transplanted limb, but it was a simple matter to test the levator response to tactile stimuli (Pringle, 1940). In a normal animal, if the dorsal surface of the tibia or tarsus is lightly touched with a paint brush, that leg is lifted and placed on top of the stimulating object. This was tried out on three of the transplanted limbs and the animals reacted by lifting the transplanted limb and placing it on the paint brush. This result suggests that the sensory fibres of the transplant have established appropriate contacts within the mesothoracic segment. This would also appear to follow from the use of the transplant in walking, to the extent that this depends on reflex activity (Wilson, 1965; Delcomyn, 1971*b*).

DISCUSSION

It is not surprising that the same identified cell body should consistently innervate the same muscle and that serially homologous cell bodies should supply serially homologous muscles. It is, after all, only another way of showing that structure and function are minutely correlated at the level of single cells and their parts. That serially homologous cells do supply serially homologous muscles is particularly evident from the results with mesothoracic cell bodies nos. 29 and 30 and metathoracic cell bodies Nos. 27 and 28; these were shown to be homologous cell bodies on anatomical grounds before it was possible to determine their function by intracellular stimulation, and so there was no possibility of the physiological data biasing the morphological assessment.

It is interesting that the pair of mesothoracic cells nos. 29 and 30, whose cell bodies are always so close together, supply muscles which are functionally interdependent. The muscles 118 and 126, supplied by cell no. 29, exert a strong adductor component on the coxa, so holding it in position while the forward locomotory thrust is delivered by the coxal depressor muscles, supplied by cell no. 30, extending the coxa-trochanteral joint (Dresden & Nijenhuis, 1953). The same obviously applies to metathoracic cell bodies 27 and 28. A similar situation occurs with the eyecup-withdrawal muscles of crabs. Several functionally interdependent muscles which withdraw the eyecup are supplied by two motor neurons (Burrows & Horridge, 1968) and the cell bodies and processes of these two motor neurons are situated very close together (Sandeman, 1971). It seems likely that this sort of situation will be found to be widespread as more examples are studied.

The fact that entire metathoracic legs can be transplanted successfully to the mesothoracic segment, where they retain their original character, confirms the conclusion reached by earlier workers on limb transplants. Bullière (1970) and Bohn (1971) in their most recent contributions both review the evidence as to whether pieces of limb retain their original character when transplanted to another segment. They are agreed that, provided it remains alive, the transplant retains its original character and will impose its regional specificity on a subsequent regenerate. The present results fully confirm these conclusions with regard to cuticular structures, but the motor nervous system behaves differently. There cannot be any barrier to motor neurons from the host segment innervating the transplant muscles and the specificity of innervation must be independent of segmental distinctions or gradients. The preliminary and circumstantial evidence suggests that the same may be true for the sensory system. This is worth looking at in more detail, especially in view of the known specificity of cockroach leg receptors at the single-cell level (Young, 1970). But so far it would appear to be the case that the individual segments are not separately numbered so far as the nervous system is concerned, whereas they obviously are so far as the cuticle is concerned.

SUMMARY

1. The connexions of single, identified motor neurons have been studied in the thoracic ganglia of the cockroach, *Periplaneta americana*. Selected cell bodies were identified on purely morphological criteria from one animal to another. Similarly, serially homologous cell bodies were identified from one ganglion to another. Their connexions to particular limb muscles were demonstrated by intracellular stimulation through the cell body and subsequent marking with procion yellow dye.

2. Serially homologous cell bodies in the mesothoracic and metathoracic ganglia innervate serially homologous muscles in the mesothoracic and metathoracic limbs.

3. Metathoracic limbs transplanted to the mesothoracic segment retain their metathoracic characteristics. They become functionally incorporated in the mesothoracic segment and are used normally during walking movements.

4. These transplanted metathoracic limbs become re-innervated from the mesothoracic ganglion. Identified mesothoracic cell bodies make specific connexions with those metathoracic muscles which are the serial homologues of their own muscles.

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EXPLANATION OF PLATES

PLATE 1

The identity of mesothoracic motor neurons 29 and 30.

(*a*) Whole mount of a mesothoracic ganglion stained with methylene blue to show the cell bodies; the stain is slightly heavier on the left. This shows clearly the position of cells 29 and 30 in the ganglion and may be compared with the cell map in Fig. 1.

(*b*) Motor neurons 29 and 30 seen in transverse section: a normal histological preparation viewed with phase-contrast microscopy.

(*c*) Cell body 29 filled with procion yellow and viewed with fluorescence microscopy: transverse section of a de-sheathed experimental ganglion.

(*d*) Cell body 30 filled with procion yellow and viewed with fluorescence microscopy: transverse section of a de-sheathed experimental ganglion.

(*c*) and (*d*) are at the same magnification.

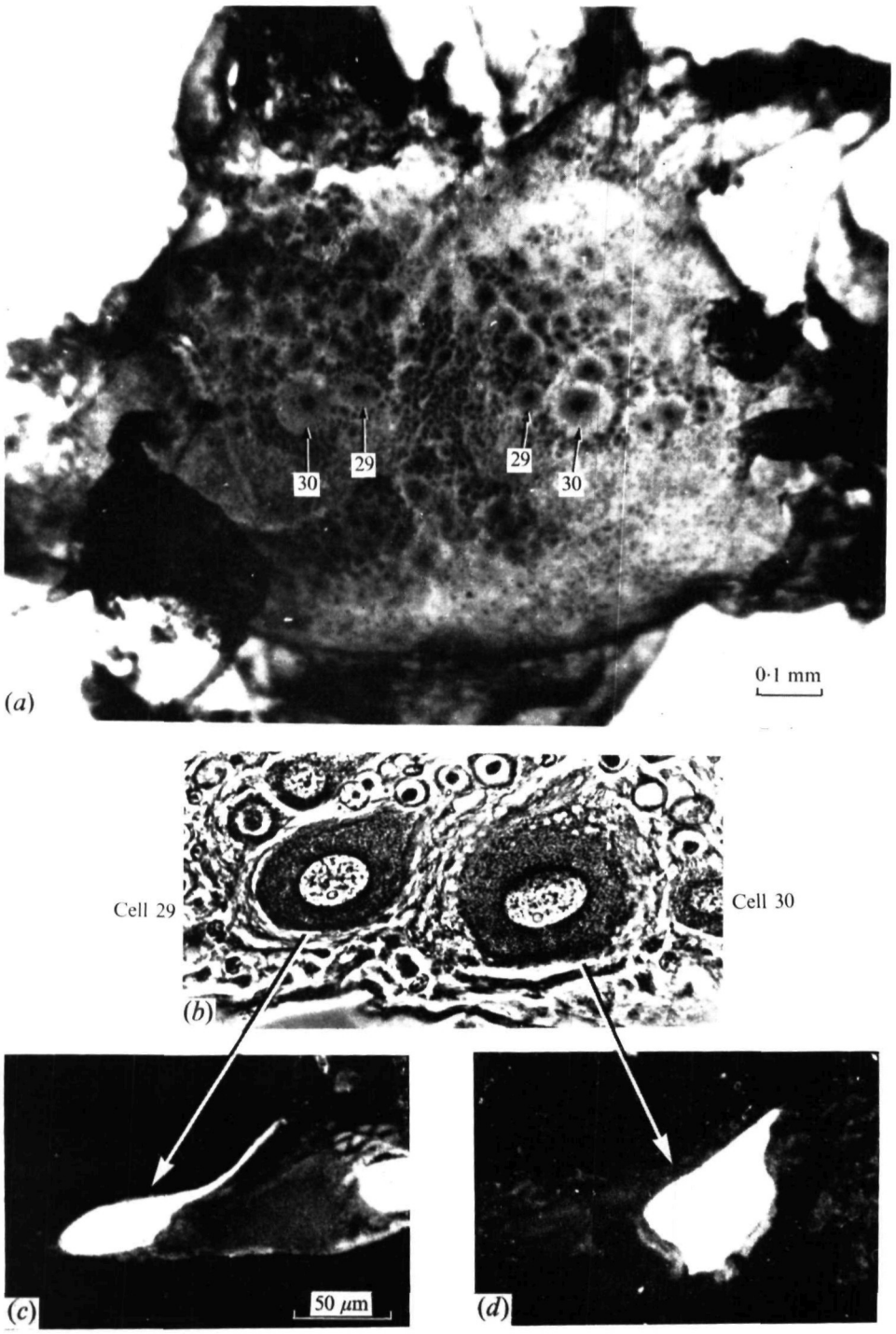
PLATE 2

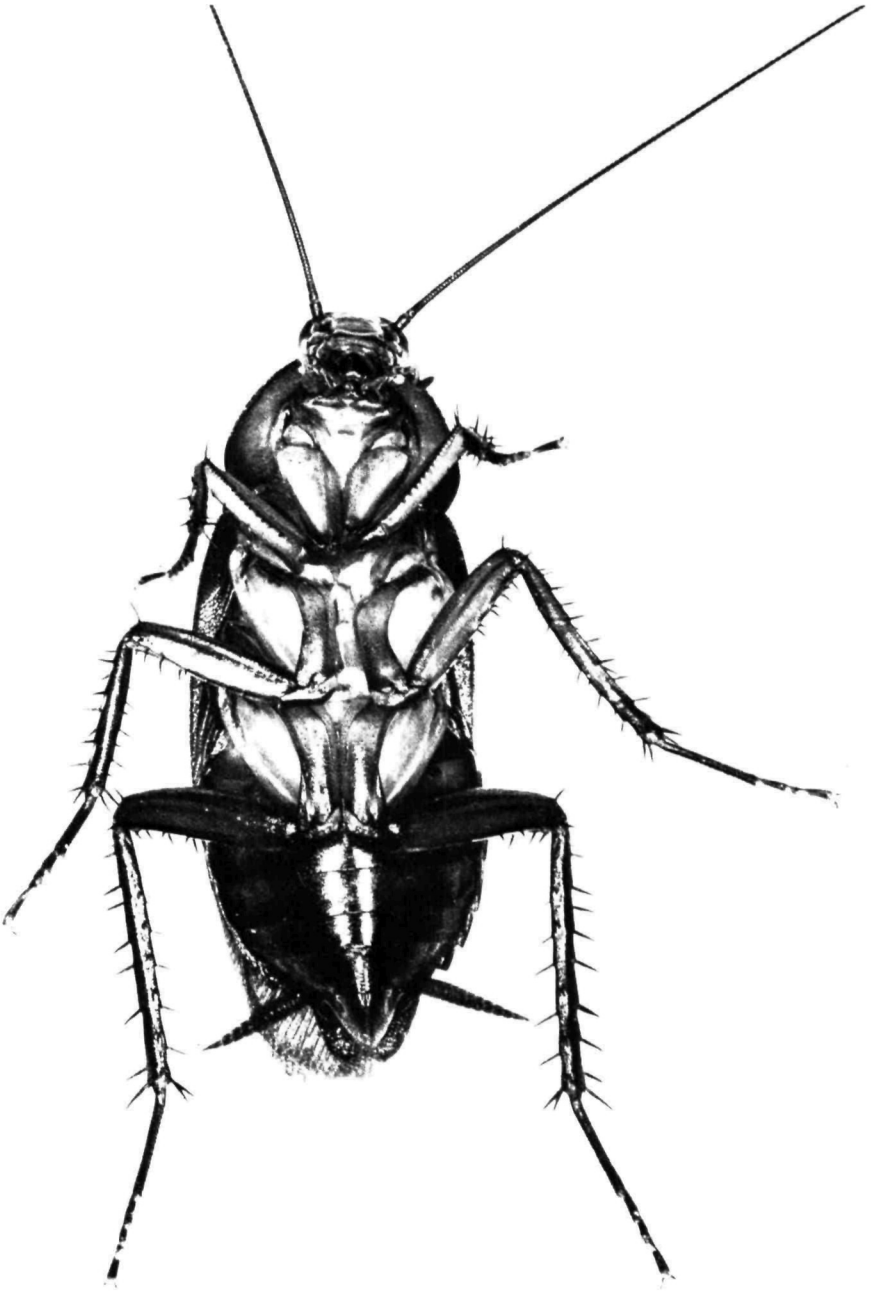
An adult female cockroach in which the left mesothoracic leg had been removed and replaced with a transplanted metathoracic leg three instars previously. Compare the transplant with the control mesothoracic and the normal metathoracic limbs especially with respect to the relative length of the femur and tibia and the number and arrangement of the tibial spines.

PLATE 3

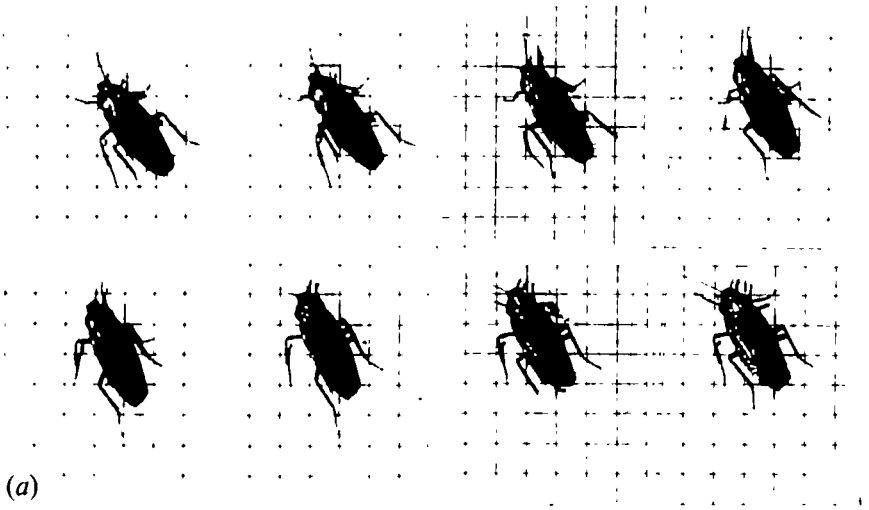
(*a*) Adult male cockroach, with transplanted metathoracic limb at L_3, walking at approximately 15 cm/s: eight consecutive frames of cine film at 64 frames/s (reading left to right). As each limb is protracted it appears blurred on the film; in the first frame L_3 is completing its protraction and R_1 is beginning is protraction. Thereafter the limbs are protracted in the normal sequence R_1, L_2, R_3, L_1, R_2, L_3. In the final (eighth) frame the animal comes to a halt.

(*b*) Tibiae from an adult transplant individual, from left to right: control mesothoracic limb, transplanted metathoracic limb, normal metathoracic limb. Note especially the relative number of fine hairs (arrowed) on each of the three tibiae.

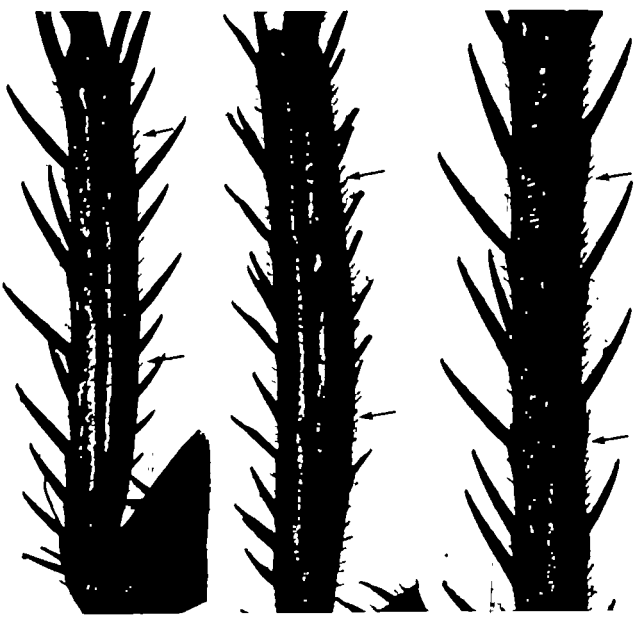

30
29
29
30
0·1 mm
(a)
Cell 29
Cell 30
(b)
(c)
50 μm
(d)



D. YOUNG



(*a*)



(*b*)