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NEURONAL HOMOLOGIES AND THE CONTROL OF BRANCHIAL TUFT MOVEMENTS IN TWO SPECIES OF *TRITONIA*

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SUMMARY

Comparative morphological and physiological studies of the branchial tuft retractor system of *Tritonia hombergi* and *T. diomedia* show a number of similarities and differences. In both species two homologous neurones in each pedal ganglion produce complete and partial contractions of the ipsilateral pinnae respectively.

Three pleural neurones cause bilateral contractions in *T. hombergi*: *L Pl* 6 and *R Pl* 5 inducing complete, and *R Pl* 6 partial, withdrawal of the tips. This differs from *T. diomedia*, where principal control is vested in the pleural giant cells.

The latency of tuft withdrawal declines and the rate of contraction increases with increasing impulse frequency in the retractor neurones, the musculature apparently responding to the second impulse of a series. After due allowance for conduction delays, only a small interval remains unaccounted for. Until electrical activity can be recorded from the musculature, the suggestion of the relay of central excitation through the peripheral plexus must be regarded as uncertain.

INTRODUCTION

Opisthobranch molluscs of the genus *Tritonia* have proved particularly suitable for studies on the neural mechanisms that underlie behaviour. Willows (1967) has described a technique for exposing and stabilizing the central ganglia so that intracellular recordings can be made from single units in whole-animal preparations of *Tritonia diomedia*, and subsequently the activity of many neurones visible on the dorsal surface of the brain has been causally related to specific facets of the animal's behaviour (Dorsett, Willows & Hoyle, 1969; Willows & Hoyle, 1969; Willows, Dorsett & Hoyle, 1973*a*, *b*). The size, pigmentation and relative position allow many of these neurones to be identified as individuals, and their physiological and functional characterization to be studied from preparation to preparation.

The morphology of the ganglia and principal nerve trunks of *T. diomedia* corresponds to that of the closely related Atlantic species, *Tritonia hombergi* (Alder & Hancock, 1909; Dorsett, 1967). Visual comparison of the neurone somata visible on the surface of the ganglia in the two species immediately suggests the existence of a number of cell homologues. These identifications are based largely on criteria of relative size and similarity of position on the ganglion surface, but in some instances the comparison is supported by details of axon distribution.

During the evolution of two morphologically similar but geographically distinct species from a common ancestral stock, selective pressures act continuously on the genotypes, adapting the structure and also the behaviour of each species to its particular environment. Presumably the modifications to the behaviour will result from changes in the set of interconnexions established in the central nervous system by the relevant neurone or group of neurones during development, and will be under direct genetic control. Recently, Bentley (1971) has shown that the calling pattern of different species of crickets is determined by genetically derived information, which specifies the output of homologous neural networks in the different genotypes.

Comparative studies on the neural mechanisms controlling the behaviour of these two species of *Tritonia* provide an opportunity to study the changes in morphology, input–output relationships and effective role of neural homologues in two species which have undergone morphological and behavioural changes during evolution, yet are sufficiently similar for detailed comparisons to show the steps by which these changes have been accomplished.

Common features of the behaviour of these two species of *Tritonia* are the withdrawal reflexes elicited by a variety of mechanical and chemical stimuli applied to the body. In *T. diomedia* a small group of identifiable neurones controls the withdrawal of the branchial tufts on one or both sides of the body (Willows, 1967; Willows *et al.* 1973*a*). This paper describes the location and properties of a functionally similar group of neurons in *T. hombergi*, and discusses their probable homologies.

MATERIALS AND METHODS

Tritonia hombergi were collected sublittorally by trawling in depths of about 40 m due west of the Great Orme in Liverpool Bay. They were kept in the laboratory under circulation and occasionally fed with freshly collected *Alcyonium digitatum*. Under these conditions animals remained healthy for several months. To record from or stimulate single neurones in the brain, whole animal preparations were made according to the method described in a previous paper (Willows *et al.* 1973*a*). Movements of the branchial tufts were monitored by arranging that these structures interrupt a narrow light beam directed at a photocell placed in the water close to the tufts. The method is similar to one described by Hoyle & Willows (1973), but it was considered that sensitivity could be improved by the use of a smaller photocell (Ferranti MS 9A) which was encapsulated and positioned close to the tuft in the water. The output from the photocell and a conventional FET-follower device, used to monitor the activity of the neurone, was recorded on a Brush 260 chart recorder. Over the middle of its range, the output of the photocell was linearly related to the area of the photocell illuminated.

Details of neuronal morphology were obtained by iontophoretically injecting neurones found to cause tuft retraction with either procion yellow or cobalt chloride, according to the methods described by Stretton & Kravitz (1968) and Pitman, Tweedle & Cohen (1971).

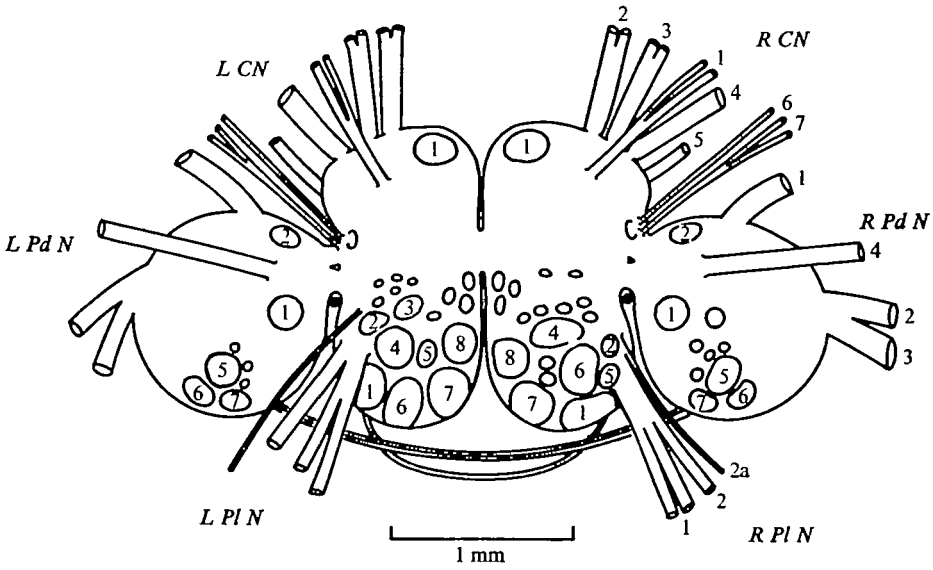

Fig. 1. Dorsal view of the brain and principal nerve trunks of *T. hombergi*, showing the position of the more obvious neurones. Numbers are assigned to cell pairs in left and right ganglia on the basis of position, appearance, morphology, activity and, where it is known, function.

RESULTS

Notation of ganglia, nerve trunks and neurones

The earlier descriptions of the brain of *T. hombergi* (Dorsett, 1967) and *T. diomedia* (= *gilberti*; Willows, 1967) revealed a fundamental similarity in the arrangement of the ganglia and nerve trunks in the two species. Subsequently it was found possible visually to recognize a number of potential cell homologues, but at that time information on the functional role of the neurones in *T. hombergi* was not available, so the establishment of homologies based on functional data could not be made.

Anticipating the requirements of future comparative studies, we have recently described a system of reference for the ganglia, nerve trunks and large somata which is applicable to both species of *Tritonia* (Willows *et al.* 1973*a*). The previous description of the ganglia and nerve trunks of *T. hombergi* (Dorsett, 1967) has been extended and revised in accordance with the principles stated in that publication (Figs. 1, 2).

Cell homologies

It is not intended at this stage to provide a detailed description of all the identifiable neurones in the central ganglia of *T. hombergi*, but it is convenient to indicate some of the more prominent neurones which appear to have homologues in *T. diomedia*. The two cerebral giant cells, *L–R Ce* 1, located on the anterior border of the cerebral ganglia (Fig. 1), are obvious homologues to a similarly placed pair in *T. diomedia*. They are the only giant cells in this ganglion and have axons in the ipsilateral buccal connective. These cells may have further homologies with similar cells found in pulmonates (Cottrell, 1971). There are five prominent neurones in each pedal ganglion of *T. hombergi*. A posterior group of three somata, *L–R Pd* 5, 6, 7 (formerly 2, 1, 3),

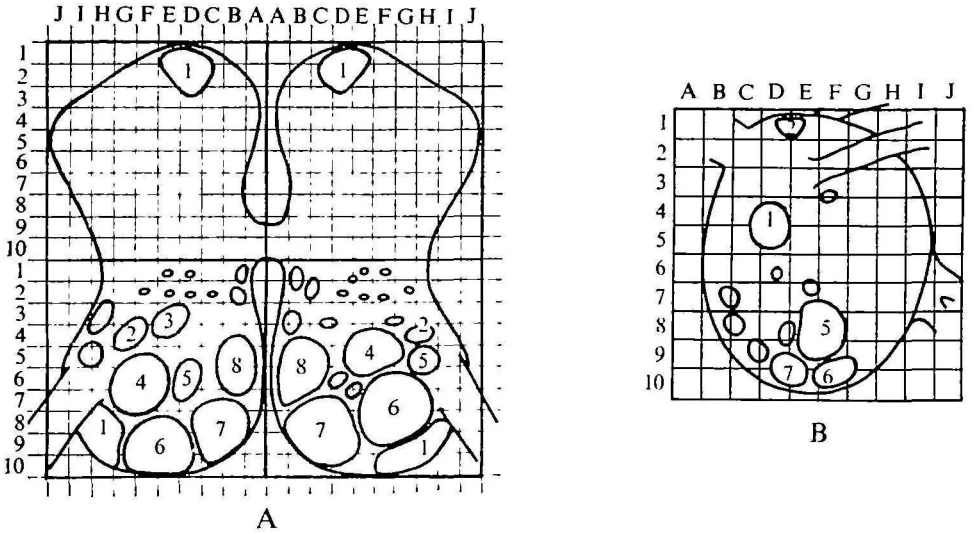

Fig. 2. The grid reference system applied to dorsal views of (A) the cerebro-pleural ganglia and (B) the right pedal ganglion of *T. hombergi*. The grid provides co-ordinates for any point on the ganglion surface, and is particularly useful for reference to small neurones and comparative studies on other species of *Tritonia*.

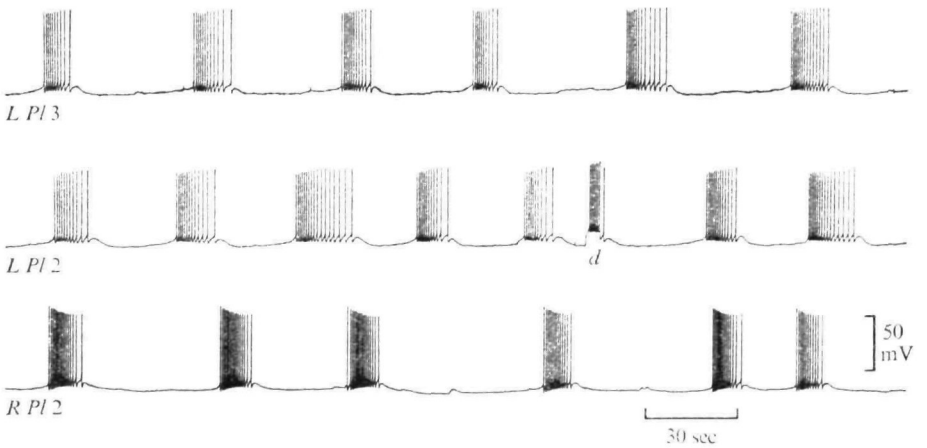

Fig. 3. Patterned bursting typical of neurones *L Pl* 2, 3 and *R Pl* 2. These neurones contain white pigment and have no obvious motor function. They are thought to be neuro-secretory. The rhythm in each neurone is endogenous and apparently independent of the others. The brief depolarization (*d*) of *L Pl* 2 resets the rhythm.

located in areas *E–F*, 8–9 (Fig. 2B), are whiter than the surrounding neurones. This, together with the fact that intracellular stimulation of these cells in whole-animal preparations produces no obvious motor effects, has led to the suggestion that these cells are neurosecretory (Willows & Hoyle, 1967). The somata of *Pd* 5, 6 are approximately 240 μm in diameter, while *Pd* 7 is normally about 200 μm. A pair of similar neurones *L–R Pd* 5, 6 having white pigmentation and no obvious motor function are found in areas *E–F*, 5–7 of the pedal ganglia of *T. diomedia*. Two other pedal neuron

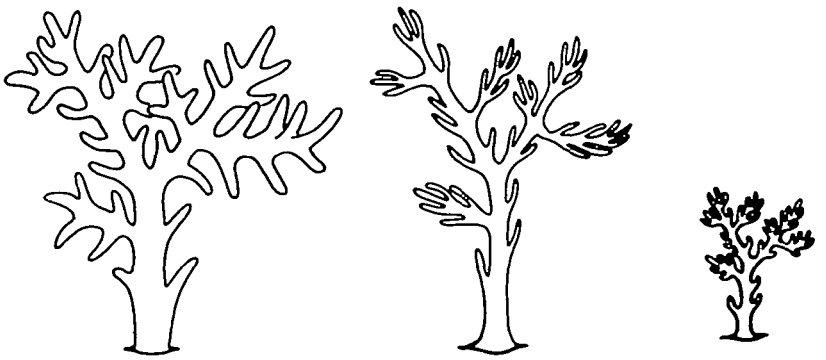

Fig. 4. The branchial tufts of *T. hombergi*. (A) Fully expanded. (B) Partially contracted, typically the result of stimulating *L–R Pd* 2 and *R Pl* 6. (C) Fully contracted, the result of stimulating *L–R Pd* 1 and *L Pl* 6 and *R Pl* 5.

L–R Pd 1, 2 participate in the withdrawal of the branchial tufts, and will be further discussed below.

Although the numbers of large neurones at the posterior end of each pleural ganglion are approximately the same, the arrangement on left and right sides is dissimilar, and contralateral homologies are difficult to establish. The arrangement represented in Fig. 1 is a generalized condition compiled from an examination of over 50 brains. The pleural giant cells *L–R Pl* 1 are an obvious pair, corresponding to two similarly placed neurons in *T. diomedia*. They are characterized by multibranched axons, distributed to nerve trunks on both sides of the body (Dorsett, 1967; Willows, 1967), but there appear to be differences in their motor function (see Discussion). Neurones *L Pl* 2, 3 and *R Pl* 2 are lighter in colour than neighbouring large cells and commonly show a spontaneous rhythm of patterned bursting (Fig. 3), and are thought to have a neurosecretory function. These neurones have their counterparts in the pleural ganglia of *T. diomedia*, which are similar in position, colour and endogenous rhythm (Willows & Hoyle, 1967).

Responses of the branchial tufts

The branchial tufts are a fringe of respiratory structures borne on the lateral margins of the mantle. Each tuft is a tubular stalk bearing a crown of about five bipinnate arms, which superficially resemble the polyps of *Alcyonium digitatum* upon which the animal feeds. There are about six pairs of dorsal tufts, spaced at regular intervals along the length of the body, and a series of laterally projecting tufts which arise from the edge of the mantle between the dorsal tufts. The tufts become smaller towards the posterior end. No physiological difference has been observed between the dorsally and laterally held types.

Individual tufts are extremely mobile, each pinna being capable of a variety of delicate withdrawal responses to gentle tactile stimulation, without affecting the other pinnae on the same stalk. Typically the pinnules shorten and fold along the axis of the stimulated pinna. Repeated mechanical stimulation of a single tuft may cause the pinnae to fold together at the tip without causing contraction of the stalk (Fig. 4B). More intense stimulation will cause the circular and longitudinal muscles

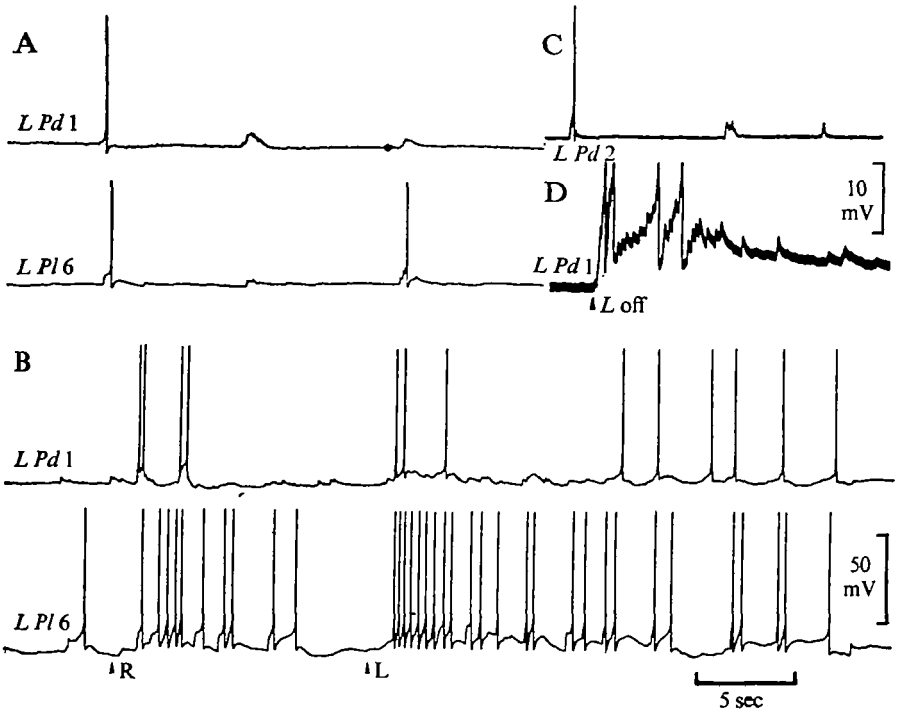

Fig. 5. Responses of the branchial tuft withdrawal neurones to various stimuli. (*A*) responses of *L Pd* 1 and *L Pl* 6 to light touches to the tufts of the left side, the oral veil, and the tufts of the right side. (*B*) The responses of the same cells to single crystals of KCl dropped on to the tufts of the right (R) and left (L) sides. Note the greater sensitivity of the pleural neurone to contralateral stimulation and the close synchronization of the later spikes. (*C*) Responses of *L Pd* 2 to water drops on the surface of the water in the aquarium in front of the animal. (*D*) Response of *L Pd* 1 to switching off the lamp illuminating the preparation. Note the prolonged e.p.s.p. input and several spikes generated in the cell. The recording is at a higher gain than the other records.

of the stalk to contract and the tuft to become small and white (Fig. 4C). All these responses can be observed in preparations from which the brain has been removed and can therefore be presumed to result from the spread of activity in a peripheral sensori-motor plexus.

Severe mechanical or chemical stimulation of localized areas of the body wall of intact preparations normally results in an integrated response involving the simultaneous withdrawal of the tufts on one or both sides of the body. Such responses cannot be obtained from decerebrate animals, since they result from the activation of specific central neurones (Willows, 1967; Willows *et al.* 1973*b*).

Neurones effecting tuft withdrawal in T. hombergi

Intracellular stimulation of the neurones *L–R Pd* 1 results in complete withdrawal of all ipsilateral tufts (Fig. 4C). These two large neurones (240 μm in diameter) are normally located in area *C–D*, 4–5 of the pedal ganglia, just posterior to the origin of nerve 4 and on a level with the statocysts. In many preparations *R Pd* 1 is not visible in this position, and in this event a similarly sized neurone, with identical motor properties, can usually be found in the right pleural ganglion area *I*–4. It is interesting

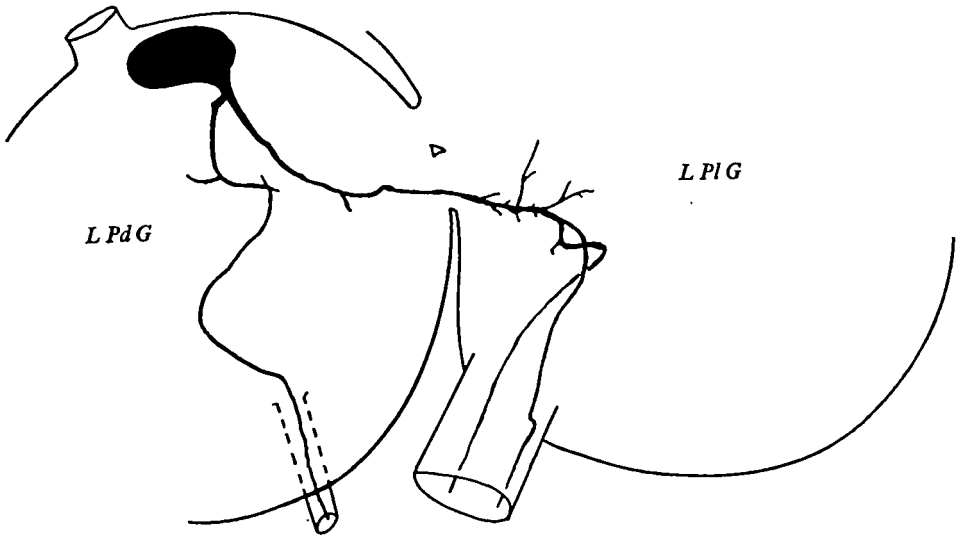

Fig. 6. Drawing of a cobalt injected preparation of *L Pd* 2. Note the fine side-processes of the axon and the branch to the pedal commissure.

to note that a similar displacement frequently occurs in *T. diomedia*. On one occasion a displacement of *L Pd* 1 occurred to position *G*, 8–9 of the left cerebral ganglion. Both these neurones have a single axon which enters the pleuro-pedal connective and turns posteriorly to enter the common base of the main pleural nerves dividing into three branches which enter *L–R Pl N* 1, 2 (Fig. 12, Plate 1). There are no obvious dendritic branches to the axon, although fine hair-like side processes arise laterally from the axon as it passes through the pleural neuropile. These processes probably make synaptic contacts with other axons (Tauc, 1962; Gorman & Mirolli, 1970; Benjamin & Ings, 1972). Occasionally, stimulation of these neurones produces variable motor effects in that only the tufts in the anterior, middle or posterior third of the body are withdrawn. These may correspond to the areas innervated by the three principal branches of the axon and represent failure of the action potential to propagate into all of the branches. On three occasions stimulation of *L Pd* 1 has resulted in withdrawal of contralateral tufts in the middle region of the body. As the axon has no branches in the contralateral nerves, it must be presumed that the responses arose as a result of excitation transmitted to another neurone. Both *L Pd* 1 and *R Pd* 1 are sensitive to mechanical and chemical stimuli (in the form of salt crystals) applied to both sides of the body. Tactile stimulation with a fine nylon filament, or water jets directed at the mantle, oral veil, branchial tufts and rhinophores, result in e.p.s.p. volleys and spikes (Fig. 5), and both neurones are sensitive to single drops of water falling on the surface of the water in the experimental chamber, several centimetres away from the animal. The neurones respond with a synaptically driven burst of impulses to an abrupt shading of the preparation.

The pair of neurones *L–R Pd* 2, visible on the anterior border of the pedal ganglia (areas *D–E*, 1), also activate the ipsilateral tufts. Intracellular stimulation of these cells causes only partial withdrawal of the tuft (Fig. 4B), the pinnae and pinnules contracting, without the stalk being visibly affected. At frequencies below 1 Hz, each

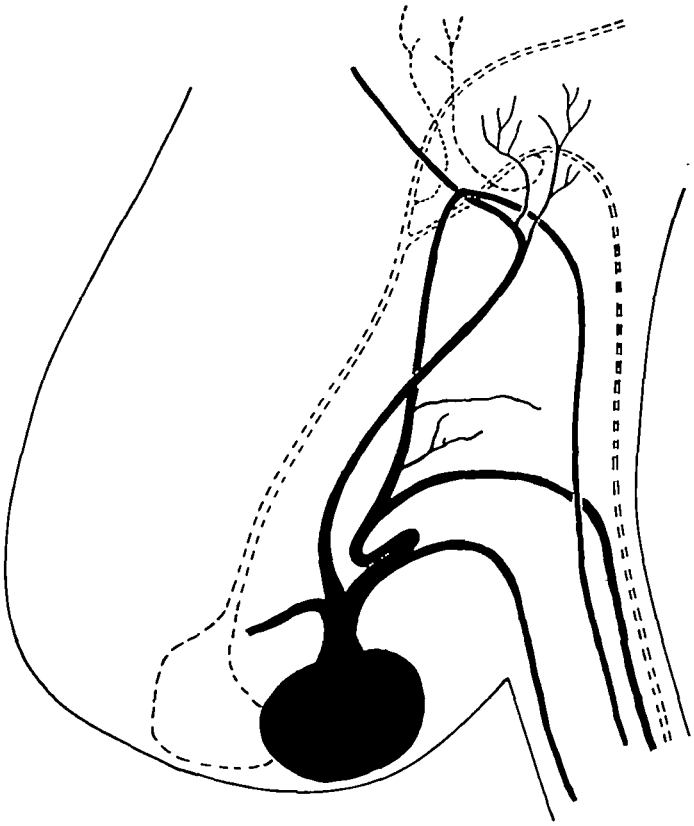

Fig. 7. Drawing of a cobalt injected preparation of *L Pl* 6 seen from the ventral aspect. The outline of the 'ghost' neurone filled by diffusion from *L Pl* 6 is shown by the dotted outline.

impulse is accompanied by a twitch, in which all the pinnae shorten and are slightly raised, but the subsequent relaxation is complete and no summed contraction results. At higher frequencies the tips of the ipsilateral tufts progressively contract until they are fully withdrawn. Each neurone is 110–150 μm in diameter, the axon dividing as it leaves the soma to send a branch into the ipsilateral pedal commisure and another into the pleuro-pedal connective (Plate 1; Figs. 6, 7). The latter branch turns posteriorly into the pleural nerve trunk, dividing again to supply an axon to pleural nerves 1 and 2. Cobalt-injected preparations reveal lateral processes arising from the axons as they pass through the pedal and pleural neuropiles, which presumably provide sites for synaptic interaction. These neurones respond to mechanical, chemical and photic stimulation in a similar manner to cell *Pd* 1, the response to water drops undergoing rapid anti-facilitation to repeated stimulation at 5 sec intervals (Fig. 5C). The response to salt crystals dropped on the oral veil is typically more prolonged.

Neurones mediating bilateral effects

The large neurones at the posterior end of the pleural ganglia typically generate co-ordinated responses involving muscle groups on both sides of the body. This property relates to the branched and bilaterally symmetrical distribution of the

axons to several of the principal nerve trunks. Branchial tuft responses are principally controlled by three neurones: *L Pl* 6, *R Pl* 6, and *R Pl* 5.

L Pl 6 is a large neurone 350 μm in diameter, located next to the giant cell in area *L Pl E–F*, 8–10. Stimulation of this cell at frequencies greater than 2 Hz results in complete bilateral withdrawal of all branchial tufts, followed after 1–2 sec by a slow swing of the tail to the left. In ageing preparations, or those subjected to prolonged stimulation, only part of the motor field may respond, or peripheral responses may fail altogether.

The axon distribution of *L Pl* 6 has been studied by electrophysiological and dye-injection techniques (Fig. 7; Fig. 13, Plate 2) and the noteworthy features are as follows:

(*a*) The principal axon passes anteriorly, turning through the cerebro-pleural commissure into the neuropil of the right pleural ganglion, where its diameter decreases and it divides into three branches, one entering the right pedal ganglion, the other two entering the right pleural nerves 1 and 2. The large diameter of the axon will ensure rapid conduction and a simultaneous spread of excitation to left and right sides of the body.

(*b*) Several small branches arise from the main axon and enter the ipsilateral pleural nerves.

(*c*) A 'ghost' cell partially fills with either procion yellow or cobalt chloride injected into *L Pl* 6, presumably by diffusion through electronic junctions, although these have not yet been established.

(*d*) Dendritic arborizations, other than slender branches of the main axon, are absent.

The neurone receives sensory synaptic input from mechanoreceptors and chemoreceptors on both sides of the body, but is more sensitive to contralateral stimulation than the ipsilateral tuft retractor neurones *L Pd* 1, 2 (Fig. 5).

Its contralateral homologue *R Pl* 6 has a similar axon distribution but depolarization only produces a partial withdrawal of the tips of the tufts on both sides of the body. This effect is similar to the unilateral response resulting from stimulation of *L* or *R Pd* 2. One of the major reasons for considering *L* and *R Pl* 6 to be homologues is the presence of the electronic junctions they both establish with the ipsilateral neurone *Pd* 2 (Dorsett & Willows, 1974).

The only neurone in the right pleural ganglion consistently causing complete bilateral withdrawal of the branchial tufts is *R Pl* 5, a cell about 160 μm in diameter located in the region *H*, 4–7 on the dorsal surface of the pleural ganglion. The position of this neurone can be variable, occasionally being identified in the area *E* 6 or sometimes not at all. The effects of stimulating *R Pl* 5 are immediate, widespread and dramatic (Fig. 8). The branchial tufts on both sides are rapidly withdrawn and the body elongates and stiffens, presumably due to increased tonus in the vertical and transverse muscles of the body wall, which becomes distinctly 'square' in cross-section. The outer margins of the foot curl dorsally and the oral veil is raised and pulled laterally, the latter effect being due to contraction of transverse muscles between the rhinophores. The oral veil responds with single twitches to impulse rates as low as 0·15 Hz in this neurone, but the branchial tufts do not retract until the firing rate rises above 1 Hz. The tufts may also show contractions to individual

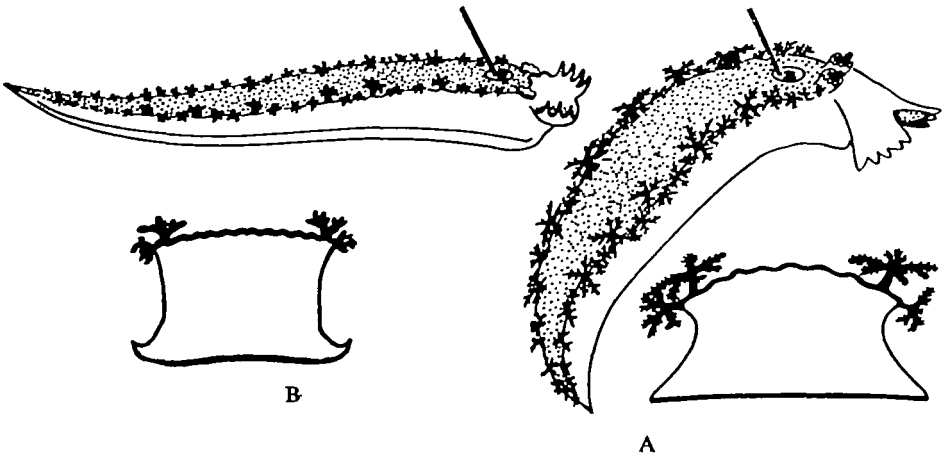

Fig. 8. Lateral views and cross-sectional representations of the body of *T. hombergi* to illustrate the effects of stimulating *R Pl* 5. (A) The preparation is relaxed with the branchial tufts and oral veil extended, the body roughly D-shaped. (B) When *R Pl* 5 is stimulated the branchial tufts contract, the oral veil is pulled laterally and raised, the body flexes dorsally, straightens and stiffens and becomes square in cross-section with the edges of the foot raised.

impulses at the lower frequencies (Fig. 11*b*), but these have proved difficult to record using the photocell technique.

Cobalt-injected preparations of this neurone (Fig. 9) reveal a complex axonal structure in keeping with the diversity of its motor activities. Four axons arise from separate points on the dorsal surface of the soma, the largest of these undergoing an almost immediate subdivision. The two largest branches pass anteriorly in the pleural neuropil, one making several loops in the vicinity of the pleuro-pedal connective, before they enter the cerebro-pleural commissure. Two of the smaller axons enter the ipsilateral pleural nerves 1 and 2 and undergo considerable branching, the nerves frequently containing more than one branch of the same axon. The remaining axon takes a median course through the pleural neuropil to pass through the ipsilateral pleuro-pedal connective. Both the large axons give rise to a number of smaller branches, the majority of which eventually enter the neuropil of the ipsilateral pedal ganglion. Some probably make the widespread synaptic contacts found between this cell and the pleural and pedal neurones (Dorsett & Willows, 1974; Willows & Dorsett, 1974), but others continue to exit in *Pd N* 2, 3. One group of four small axonal branches passes through the cerebral neuropil, presumably innervating the musculature of the oral veil through cerebral nerves 2 and 3. Unfortunately, in all iontophoretically injected preparations of this neurone obtained to date, the stain has failed to diffuse far beyond the point where the axons enter the cerebro-pleural commissure to reveal the axon distribution on the left side. However, the symmetry of the muscular responses would suggest that the distribution on the left side is similar to that of the right.

The physiology of tuft withdrawal

The delicate withdrawal movements made by individual tufts in response to gentle tactile stimulation never result from direct excitation of relevant central neurones, and it is assumed that they depend on excitation arising in a localized

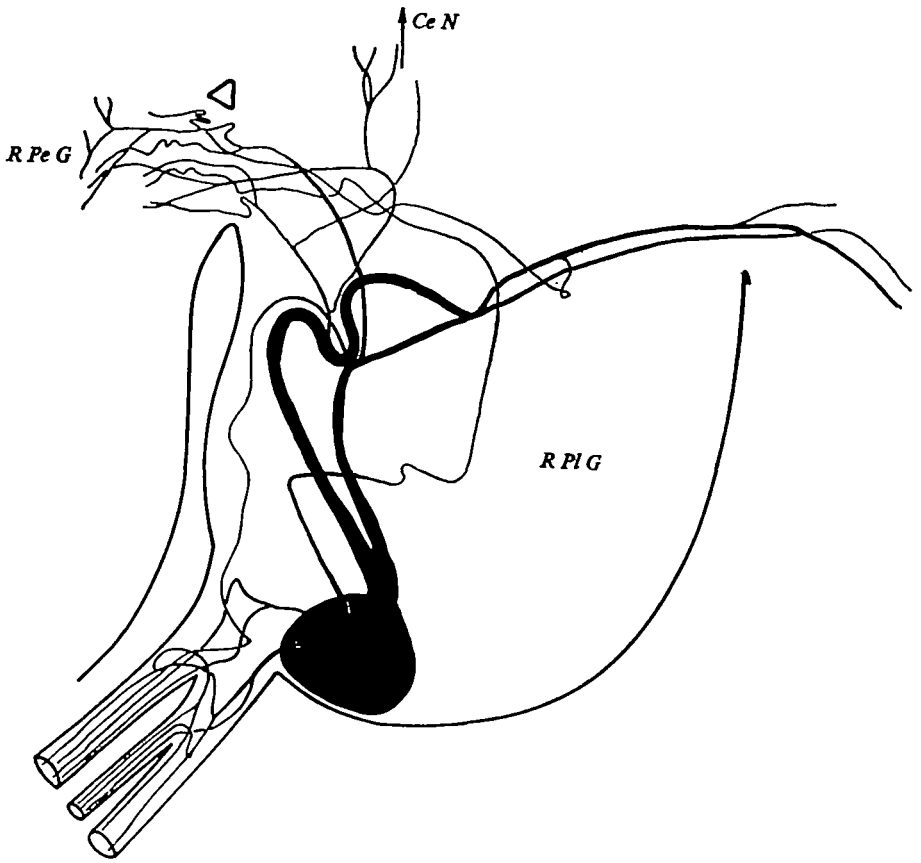

Fig. 9. Ventral view of a cobalt injected preparation of *R Pl* 5. The five axons arise from four separate points on the soma. The smaller axons divide extensively in the base of the right pleural nerve trunks, and several branches from the same axon may enter a single nerve. The arrow indicates axons passing anteriorly to the cerebral nerves.

peripheral plexus. The response to a stronger stimulus is compounded from an initial local response which is subsequently reinforced, after an appropriate delay, by excitation from the efferent fibres of the central branchial tuft neurones.

The branchial tufts of debrained animals respond to single electric shocks delivered to the end of a pinna of a single tuft, and of an intensity just sufficient to cause a response, by a contraction which affects the whole tuft. The contraction has a latency which varies between 50 and 70 msec (Fig. 10). The area responding is too widespread to result from direct excitation of the muscle fibres, and presumably results from the activation of sensory fibres in the reflex pathways of the peripheral plexus.

The simultaneous withdrawal of branchial tufts obtained by stimulating *L–R Pd* 1, *L Pl* 6 or *R Pl* 5 typically has a much longer latency, which consists of the conduction delay to the periphery and another delay which is dependent upon the impulse frequency in the central neurone (Fig. 10B, C). The branchial tufts normally show no response to impulse frequencies below 1 Hz, but firing rates above 2 Hz are normally adequate to bring about sustained withdrawal of the tufts. At this frequency *L Pl* 6

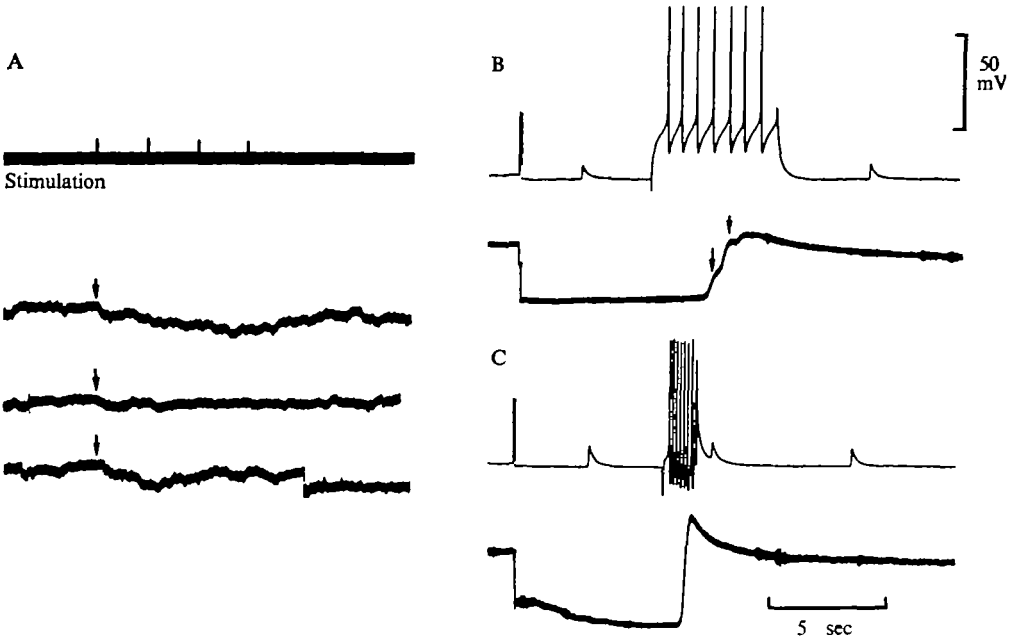

Fig. 10. (A) Responses of the branchial tufts, recorded as the output from a photocell, to direct stimulation of a pinna through a suction electrode. Latency is about 50–60 msec. (B), (C) Responses of a branchial tuft to intracellular stimulation of the tuft retractor neurone *R Pl* 5 at two different frequencies. Both the latency and the rate of the response depend upon the impulse frequency in the neurone. In B the arrows indicate twitches corresponding to individual impulses in the neurone.

causes a bilateral withdrawal commencing after a latency of about 2 sec, 10–12 impulses being required to bring about complete withdrawal of the tufts in the posterior two-thirds of the body, and a further eight impulses to pull in the anterior tufts. The movements are accompanied by a slow swing of the tail to the left.

With increased firing rates the total latency falls until at frequencies of 7–8 Hz, which is near the maximum these neurones can be driven by depolarization through the electrode, it approaches a minimum value of 350–400 msec. The data presented in Fig. 11 (A) represent the pooled results of five experiments on *L Pd* 1, *L Pl* 6 and *R Pl* 5, all of which when plotted individually appeared to fit the same curve. Each point represents the mean of the minimum latency measured for each neurone at that particular impulse frequency. The conduction velocity of the action potential of these neurones measured over 3 cm of the pleural nerve trunks is approximately 20 cm/sec, which in practical terms means a delay of 150 msec between the peak of the action potential in the soma and its arrival at the point where the pleural nerves enter the body wall in the middle region of the body.

The figure also includes a plot of the impulse interval at the chosen frequencies, which lies parallel to but is separated from the total latency curve by an interval of about 200 msec down to impulse frequencies of 2 Hz. Below this value the latency becomes significantly prolonged. If one assumes that the first impulse in the series is ineffective, the results suggest that the response latency follows the curve representing the time of arrival of the second impulse plus the sum of the conduction delay and delays due to interaction with the peripheral motor plexus or at neuro-muscul

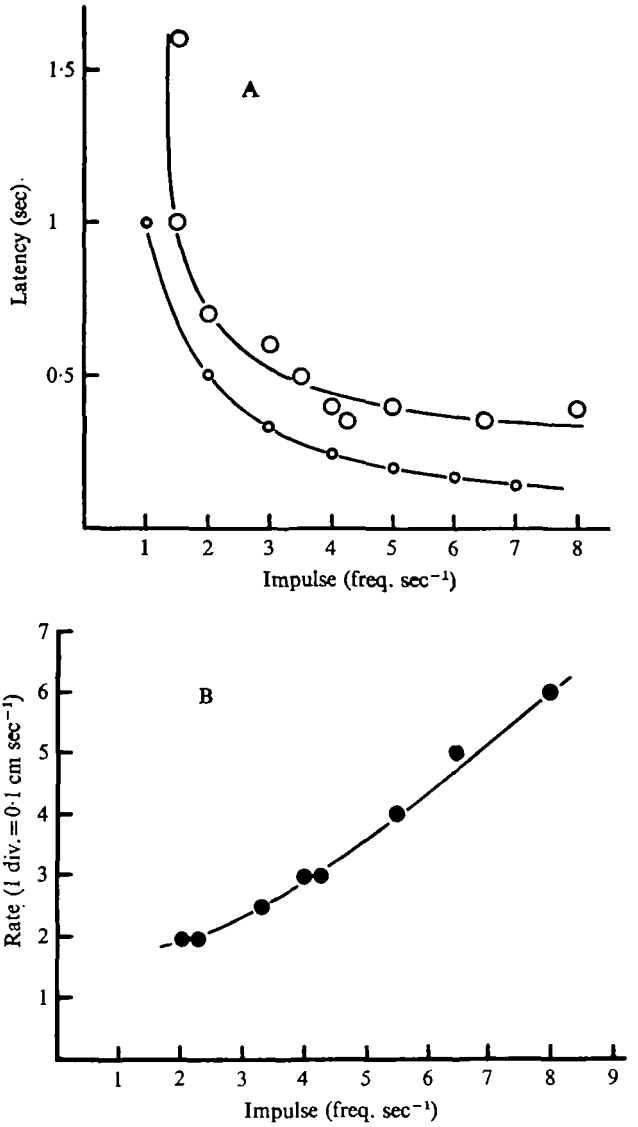

Fig. 11. (A) The relationship between latency and impulse frequency in the retractor neurones. Large circles: average minimum latency at different frequencies measured in five experiments involving *L Pd* 1, *L Pl* 6 and *R Pl* 5. Small circles: the impulse intervals at the given frequencies. From 2–8 Hz the latency is about 200 msec relative to the initiation of the second impulse in the neurone. (B) Relationship of the rate of tuft withdrawal to impulse frequency.

junctions. Anomalies at frequencies below 2 Hz could result from the temporal properties of facilitation at the more peripheral junctions.

The rate of tuft withdrawal also depends upon the impulse frequency in the tuft retractor neurones (Fig. 11B). The maximum rate of movement at impulse frequencies of 7 Hz represents velocities between 0·2–0·35 cm sec^{-1}. This result also suggests that facilitation may occur at the neuro-muscular junction.

DISCUSSION

Neural homologies in the tuft retractor system

The co-ordinated withdrawal of the branchial tufts of *T. hombergi* may be accomplished by stimulation of any one of seven neurones on the dorsal aspect of the central ganglia. Two symmetrical pairs of pedal neurones *L–R Pd* 1, *L–R Pd* 2 control the unilateral responses: the former produce complete retraction, and the latter only withdrawal of the tips of the tufts. Despite the tendency for *R Pd* 1 to be found in the right pleural ganglion, the morphology, axon distribution and motor responses indicate that each member of these two pairs may be considered as the contralateral homologue of the other.

These neurones correspond in position and axon distribution to similar pairs in the pedal ganglia of *T. diomedia* (Willows, 1967) and have consequently been given the same numbers. Although the morphological detail obtained from dye-injected preparations is lacking in this species, and the functional differences have not been noted, there is little doubt that the homologies can be extended across the two species.

Simultaneous bilateral withdrawal of the tufts is, characteristically, a property of pleural nerones, and it is here that the most fundamental divergence is found between the two species. In *T. diomedia* only the giant pleural neurones (*L–R Pl* 1) reliably cause bilateral tuft withdrawal (Willows *et al.* 1973*a*; Hoyle & Willows, 1973) although some small cells bordering the trigger groups may occasionally do so. The giant cells in the pleural ganglia of *T. hombergi* have never been observed to produce this response, but rather they retract the rhinophores and cause contractions in the longitudinal muscles that result in the body twisting to left or right. Responsibility for bilateral branchial tuft retraction in *T. hombergi* has been subdivided between three neurones. The neurone *L Pl* 6 causes complete withdrawal of all branchial tufts, but the corresponding neurone in the right ganglion, identified by its morphology and the ipsilateral electrotonic junctions established with the neurones *Pd* 2, produces only a partial withdrawal. Complete withdrawal is commanded by *R Pl* 5, together with a number of other widespread muscular effects which have their closest parallel in the elongation of the body, and in the paddle formation that occurs in the initial stages of the swimming behaviour of *T. diomedia*. In this species the physical effects coincide with the activity of the trigger groups and other small neurones in the pedal ganglia, and so far, no homologue to *R Pl* 5 has been located. So far, *R Pl* 5 has not been implicated in the swimming behaviour of *T. hombergi*.

The physiology of tuft retraction

The analysis of the relationship of the activity in central neurones to the responses of the peripheral musculature has been handicapped by the inherent difficulties of making electrical recordings from many important muscle groups. The interpretation of the recordings of physical movements is complicated by the existence of the peripheral sensori-motor plexus, whose contribution to any observed response cannot be accurately measured. Electrical recordings have been made from a few anatomically distinct muscles with parallel fibres (Twarog, 1967; Mellon, 1968; Kater, Heyer & Hegmann, 1971) which indicate direct innervation of the muscle fibres, and a similar type of innervation was found in the gill muscles of *Aplysia* (Kupfermann *et al.* 1970).

However, an analysis of the neuro-muscular responses of the body wall and branchial tufts of *T. diomedia* led Hoyle & Willows (1973) to suggest that the activity of central neurones was relayed to the musculature by the peripheral motor network.

The results of a comparable series of experiments on the neurones controlling tuft withdrawal of *T. hombergi* reveal several differences from those obtained for *T. diomedia*. Although the minimal response latency to direct stimulation of the peripheral reflex loop in debrained preparations is similar at about 50–70 msec, the lower figures for conduction velocity in the axons of the retractor neurones of *T. hombergi* substantially reduce the delays which have been associated with the peripheral synaptic interactions.

The response latency of the branchial tufts of *T. hombergi* to central stimulation closely follows the inter-impulse interval, down to frequencies of about 2 Hz, suggesting that over the measured range of impulse frequencies the muscles are responding to the arrival of the second impulse. The total delay of approximately 200 msec is compounded of a measured conduction delay of 150 msec to the point where the pleural nerves enter the body wall, plus a conservative estimate of about 30 msec for transmission along the finer branches to the vicinity of the muscle fibres. This leaves about 20 msec to be accounted for by possible delays at junctions with the peripheral network and the muscle fibres, which is very much less than the figure of 160 msec obtained by Hoyle & Willows (1973) for *T. diomedia*.

The synaptic delay at peripheral neural junctions of *Spisula* was found to be 3·2 msec (Prior, 1972) and it is doubtful if the delay at the neuro-muscular junction of *Heliosoma* exceeds this (Kater *et al.* 1971). In view of the likely errors in the various approximations, one wonders whether it is necessary in *T. hombergi* to postulate the interpolation of the peripheral motor plexus between the muscle fibres and the central neurones. Although the alternative explanation necessitates the ultimate branches of the axon of a single neurone innervating a large proportion of the muscle fibres in all the tufts, at times a 1:1 correspondence between individual impulses in retractor neurones and twitches in the tufts can be observed. In this instance, the peripheral plexus could only be acting as a simple relay.

The other argument quoted in support of the participation of the peripheral plexus is the variability frequently encountered in the muscular response to identical central stimulation. The suggestion is made that the ultimate response then depends on the balance of excitatory and inhibitory influences existing in the peripheral plexus at that instant. It has previously been assumed that intracellular stimulation of a single neurone does not activate parallel pathways to the same system of effectors. Many previous attempts to demonstrate such interactions in *T. diomedia* have proved largely negative, but recently evidence has been obtained of polysynaptic excitatory pathways between branchial tuft retractor neurones (Dorsett & Willows, 1974) which indicate that interactions of this type may account for some of the variability encountered in the observed responses, by exciting other neurones participating in the same motor system.

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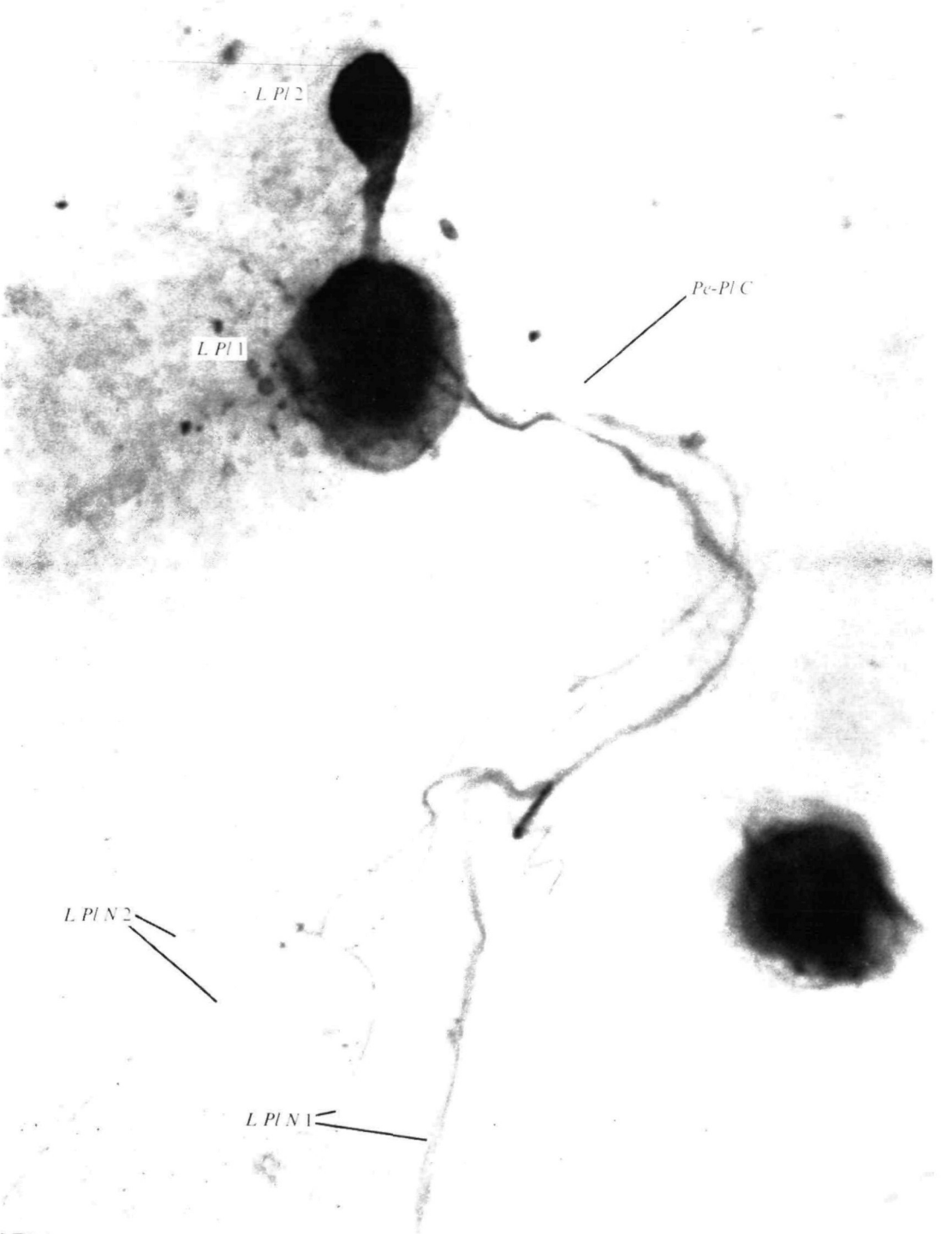
EXPLANATION OF PLATES

PLATE 1

Fig. 12. Cobalt injected preparation of neurones *L Pd* 1 and *L Pd* 2 viewed from the dorsal aspect. The axon of *L Pd* 2 passes behind *L Pd* 1, but there are no obvious points of close contact between them. *L Pd* 2 has another branch which passes ventrally into the pedal commissure, which has not filled in this preparation. The finer branches of the axons have been slightly re-touched where they pass out of the focal plane of the photograph. *Pe-Pl C*, pedal–pleural connective.

PLATE 2

Fig. 13. Photograph of a procion-yellow-stained preparation of *L Pl* 6 from the ventral side. Note the large-diameter axon passing to the right side, and the 'ghost' cell again filled by diffusion from the injected neurone. The identity of the numerous fine branches is difficult to resolve, but there is general correspondence to the morphology represented in Fig. 8. Note the numerous fine branches entering the ipsilateral pleural nerve trunk. The outline of the soma of *L Pl* 6 has been dotted in.



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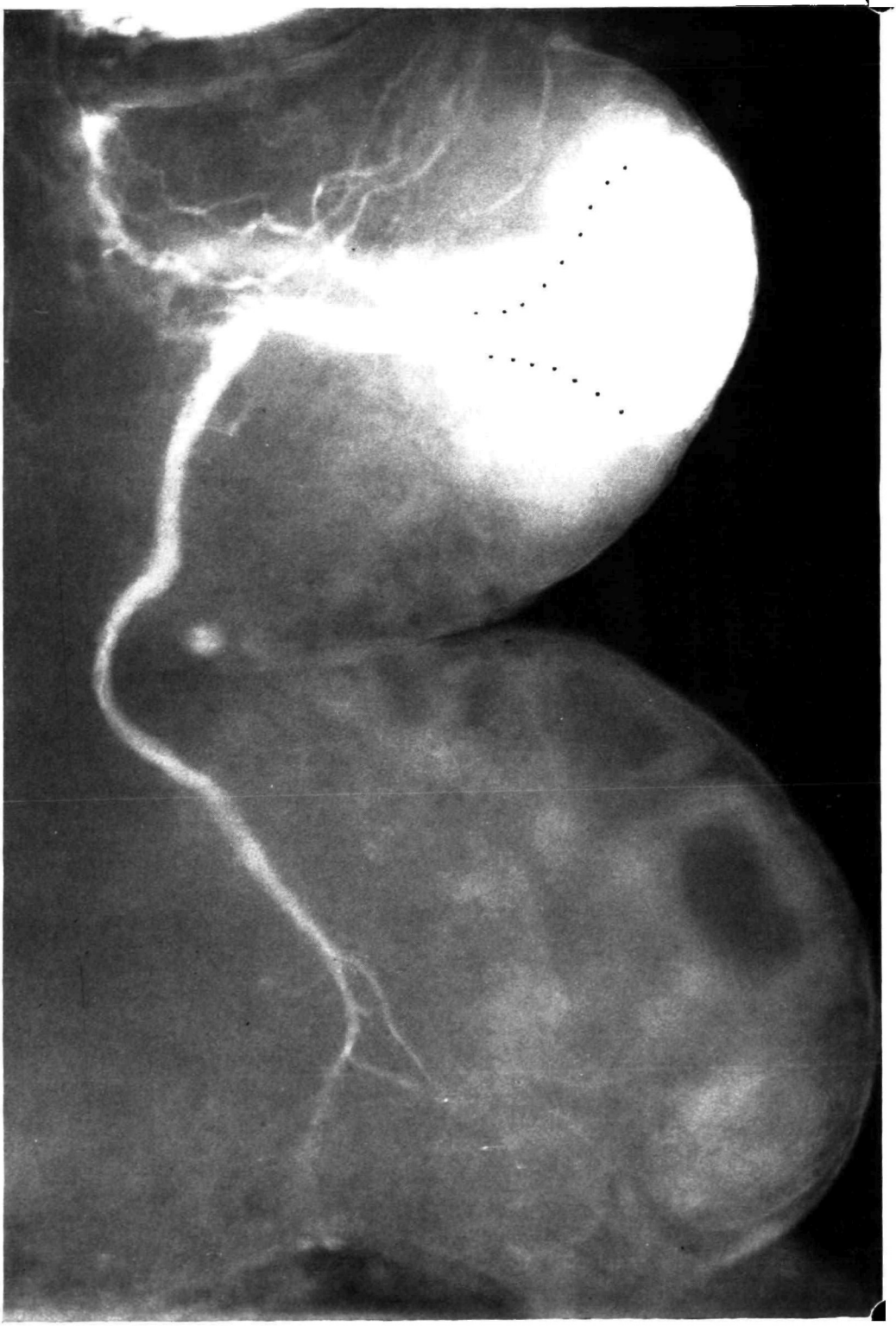
(Facing p. 654)



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