Central Neuronal Projections and Neuromuscular Organization of the Basal Region of the Shore Crab Leg

M. BEVENGUT, A.J. SIMMERS, AND F. CLARAC
Laboratoire de Neurobiologie Comparée, 33120 Arcachon, France

ABSTRACT

The musculature and associated skeleton, peripheral nervous system, and central projections of motor and sensory neurones of the two basal (thoracic and coxal) segments of the shore crab leg (fifth pereiopod, P5) were examined in vivo and with methylene blue or cobalt staining.

Each of the four main basal muscles, promotor/remotor, levator/depressor, controlling the thoracico-coxal (T-C) and coxo-basal (C-B) limb joints, respectively, comprises several more or less discrete fibre bundles (total 14), with little morphological segregation of different functional groups.

The innervation to the basal leg region is carried in two nerve roots arising from the thoracic ganglion. The anterior Th-Cx root carries both sensory and motor axons, while the posterior Th-Cx root is purely motor. Three previously undescribed sensory branches (two "epidermal" nerves and an "accessory" branch), in addition to that innervating the coxobasal chordotonal receptor, have been found in the distal part of the anterior Th-Cx root. Two clusters of 10 to 15 multipolar somata (diam. 30–125 \( \mu \)m) are located proximally at the bifurcation of the accessory nerve and distally where the latter enters the basipodite.

The cell bodies (diameter 20–80 \( \mu \)m) of basal leg motoneurones (total ca. 30) lie in the dorsal cortex of the ganglion, with somata of functionally related motoneurones tending to form discrete structural groups. The morphology of individual motoneurones conforms to the general arthropod pattern. All are confined to the ipsilateral hemiganglion and their main neuropilar processes run parallel and in close apposition to each other with overlapping dendritic structures. Sensory projections arising from the CB chordotonal organ also ramify in the region of the neuropile invaded by motoneurones. The possible physiological significance of such structural associations within the CNS is discussed, as are the functional implications of basal limb anatomy in general.

Key words: crab pereiopod, thoracico-coxal musculature, motor/sensory neurones, thoracic ganglion, neuropile

The neuronal mechanisms underlying patterns of motor behaviour in arthropods are major concerns of current neurobiological research. One system which provides a suitable model for the study of the neural organization of motor control in general is the walking system of decapod Crustacea. We are now beginning to understand some of the basic features of the central nervous coordination and regulation of this behaviour (Evoy and Ayers, '82), and there appear to be organizational principles of crustacean locomotion that are common to other invertebrate and vertebrate locomotory systems (e.g., Ayers and Davis, '77; Stein, '77). However, a necessary prerequisite for understanding central mechanisms of control is a precise knowledge of the peripheral innervation, musculature, and proprioceptive machinery associated with the moving appendage itself. Since the early anatomical work on Cancer (Pearson, '08) and Callinectes (Cochran, '35), the decapod leg has been studied extensively and there is now considerable information, both anatomical and physiological, on various aspects of its neuromuscular and mechanical organization. Most descriptions to date have concentrated on the neuromusculature of the more distal limb segments, or on the rich variety of sensory structures distributed throughout this highly complex and multifunctional ap-

Accepted June 9, 1983.

© 1983 ALAN R. LISS, INC.
pendage. The peripheral motor innervation patterns of the distal limb muscles are known from the pioneering work of Wiersma and collaborators (see Wiersma and Ripley, ‘52; Wiersma, ‘61), while a number of studies have established the functional anatomy (e.g., Alexandrowicz and Whitear, ‘57; Wales et al., ‘71) and physiological properties (e.g., Bush, ‘62, ‘65a, b; Evoy and Cohen, ‘59) of various sensory receptors and their role in reflex regulation (see reviews by Mill, ’76; Clarac, ‘77; Bush and Laverack, ‘82).

Despite the large amount of existing information on the periopods of decapod Crustacea, several important features remain undescribed or unknown in great detail. The present study was motivated by the lack of comprehensive anatomical data concerning the most proximal part (thorax and coxopodite) of the crustacean leg. This lack of information is somewhat surprising since the proximal limb region contains two separate joints, the thoracico-coxal (T-C) and coxo-basal (C-B) joints, which are functionally extremely important in both locomotion and posture, corresponding to the shoulder or hip joints of mammalian quadrupeds. The basal area of the decapod leg has been partly described by McVean (‘73) in an analysis of limb autotomy in the shorecrab *Carcinus*, and by White and Spirito (‘73) and Hoyle and Burrows (‘73) in studies on the neuromuscular physiology of two portunid crabs, *Callinectes* and *Portunus*, whose fifth periopods are specialized for swimming. But apart from other work on specific proprioceptors such as the thoracico-cocxal (TC) muscle receptor (Bush, ‘77) and the coxobasal (CB) chordotonal organ (Bush, ‘65b), the basal part of the limb has received little general attention in comparison to the more distal segments.

Thus, the intent of this report is to provide an integrated anatomical picture of the thoracic and coxopodite region of the fifth periopod of *Carcinus*, appraising its muscular organization and its overall sensory and motor innervation as a background to further functional investigation of crab leg movements. In addition, the location and central projections of basal limb motoneurones and some sensory neurones within the thoracic ganglion are described. It is intended that possession of such topographical data will further extend the basis for direct physiological study on the neural control of locomotor activity within the central nervous system itself.

**MATERIALS AND METHODS**

Shore crabs, *Carcinus maenas*, were obtained locally and kept in tanks of running oxygenated seawater. For all experiments, the nervous system and skeleton of the thorax were exposed by removing the dorsal carapace (along with the brain), heart, viscera, and gills. The preparation was then pinned down, dorsal side uppermost, for gradual removal of endophragmal skeleton and overlying thoracicococxal musculature to expose the appropriate nerves and muscles of the fifth periopod. Standard histological techniques were used in conjunction with anatomical descriptions of freshly dissected tissue.

**Methylene blue staining**

Nervous tissue was stained by immersing dissected preparations overnight at 4°C in a few drops of 1% methylene blue solution added to *Carcinus* saline (500 mM NaCl; 12 mM KCl; 12 mM CaCl2; 20 mM MgCl2; 4.3 mM maleic acid; 9.9 mM TRIS buffered to pH 7.2 with 2 M maleic acid). Stained tissues were then fixed for 6-12 hours in saturated ammonium molybdate in distilled water. After rinsing and dehydration in an alcohol series (75%, 95%, 100% at 15 minutes each), the material was cleared in toluol or methyl salicylate before observation with light microscopy and subsequent photography.

**Coat staining**

The central and peripheral projections of neurones were visualised by the migration of cobalt ions for 4-48 hours through the cut ends of selected nerves which had been immersed in a vaseline well containing 8.5% cobaltous chloride (Pitman et al., ‘73). To precipitate the cobalt ions in filled neurones, the preparation was first washed in saline, then placed in fresh saline containing 1% ammonium sulphide for 5-15 minutes. After a further wash, the preparation was dehydrated and then cleared in methyl salicylate. Stained cells were either sketched with a camera lucida or photographed in whole mount.

**Nerve cross sections**

Nerves dissected free were fixed in formaldehyde-glutaraldehyde using the Karnovsky method (‘65), postfixed with 1% osmium tetroxide, and embedded in epon-araldite. Transverse nerve sections 1 pm thick were cut on an ultramicrotome, collected on a drop of water on a microscope slide, then dried down on a hot plate at 60°C. The sectioned tissue was stained using a 0.5% methylene blue, 0.5% azure blue in a 0.5% borate solution. The sections were then observed under a light microscope and photographed.

**RESULTS**

In the shore crab *Carcinus maenas*, as in other decapod Crustacea, the chelae and walking appendages (periopods 1-5) are each composed of six segments. The most proximal limb segment, the coxopodite, articulates to the thorax, while the adjoining two segments—the basipodite, on which there exists a preformed breakage plane for limb autotomy, and the ischiopodite—are fused to form a single segment, the basi-ischio-podite. Attached to the latter is the longest limb segment, the meropodite, followed distally by the carpo-, pro-, and dactylo-podites, respectively. These last two segments are modified to form the chelicerae on the first pair of periopods (P1) while on the fifth pair (P5) in some brachyuran species, they are flattened to form a paddelike structure for swimming. The joint between adjacent pairs of segments imposes movement in a single plane about the axis of two condyles, generally in either the anteroposterior or dorsoventral directions. The alternating order of these two types of joints in each periopod thus allows movements of the limb virtually in every direction.

**Basal limb anatomy**

The skeletal system. The structure of the thoracic skeleton is such that each thoracic segment (corresponding to the five bilateral pairs of periopods) forms a skeletal box whose internal surfaces and planes are designed to provide attachment for the basal muscles of the limb (Cochran, ‘35; McVean, ‘73; White and Spirito, ‘73; Secretan, ‘80).

The internal skeletal arrangement of each thoracic partition consists of infoldings of two main exoskeletal components—the sternite (st.) and the pleurite (pl.; Fig. 1b). The hemisternites of the two posterior legs (P5) meet at the midline of the ventral thorax and invaginate in an internal...
Fig. 1. a. The thorax (dorsal view) of *Carcinus maenas* after removal of the carapace, viscera, and gills. The basal thoracic and coxal muscles of the five autotomised pereiopods (P1–P5) lie in segmental skeletal capsules. Each limb is innervated by separate nerve roots arising from the fused thoracic ganglia (TG). AN, abdominal nerves; COC, circumoesophageal connectives; SA, sternal artery. Scale = 1 cm. b. Ventral (i) and dorsal (ii) views of the basal region of the right fifth pereiopod (P5), including the thoracic portion and the three most proximal limb segments; coxopodite (COXO), basi-ischiopodite (BASI-ISCHIO), and meropodite (MERO). Arrows A–A' denote the preformed autotomy plane. The thoracic skeletal elements labelled are: i. er. pl., interopleurite; i. er. st., interosternite; md. i. er. st., medial interosternite; md. s., median structure; pl., pleurite; st., sternite.

vertical plane, the medial interosternite (md. i. er. st.). Posteriorly, the hemisternite of each P5 curves dorsally to form a gutter which provides the posterior endoskeletal wall of both the P5 appendage and the thorax as a whole (Fig. 1bii). The dorsal margin of this gutter is linked distally to the pleurite (pl.) and proximally to the median structure (md. s.), a horizontal endoskeletal plate in the rear of the thorax. The anteroventral edge of the hemisternite extends dorsally to form the interosternite (i. er. st.; Fig. 1bii), which interfaces with the posterior interosternite of the preceding (fourth) segment. The dorsal endoskeletal surface of each thoracic segment is similarly formed by the pleurite. For P5 this extends ventroanteriorly via the interopleurite (i. er. pl.) to adjoin its corresponding interosternite, again in close apposition to the posterior interopleurite of the next segment (Fig. 1bii).

The exoskeleton of the adjoining limb segment, the coxopodite, has the form of an asymmetric ring which is wider ventrally than dorsally, while the structure of the next distal segment, the basipodite, also forms a ring but is more symmetrical and smaller in diameter.

Musculature

Functional organization. The basic muscular organization of Crustacean appendages is simple; each limb seg-
Fig. 2. Muscles of the thorax and coxopodite of the fifth pereiopod (P5). The in situ disposition of muscle bundles is illustrated by the dorsal view in a, while the exploded view in b shows the number, origins, and insertions of bundles belonging to each of the four main functional muscle groups. Dotted lines indicate the axes of the thoracico-cotal (T-C) and coxo-basal (C-B) joints, while the arrows denote anterior (ant) and distal (dist) directions. REM, remotor bundles (1) posterior (P) and (2) anterior (A); DEP, depressor bundles (3–6); LEV, levator bundles (7–10) anterior (A) and (11, 12) posterior (P); PRO, promotor bundles (13, 14).

Gross anatomy of basal limb muscles. Underlying the above functional picture, however, there exists a far more complex anatomical organization of the basal limb musculature. This derives largely from the fact that each "functional" muscle (i.e., promotor, remotor, levator, depressor) comprises several discrete bundles of muscle fibres which may have completely different sites of origin and even, in some cases, separate points of insertion. Furthermore, while some smaller bundles of the levator and depressor muscles operating the second limb joint (C-B) lie wholly within the coxopodite, the larger bundles originate more proximally, along with the T-C muscles (promotor, remotor), within the thorax itself. A more detailed description of the basal P5 musculature in Carcinus is therefore presented here and is used as a basis for comparison (see Discussion) with another brachyuran species, Callinectes sapidus, whose fifth pereiopods are adapted specifically for swimming behaviour (White and Spirito, '73).

The "in situ" organization of the various basal muscles is shown in Figure 2a and represented schematically in Figure 2b. The total musculature is comprised of fourteen fibre bundles each of which has its insertion on an apodeme attached to the proximal skeletal border of the next distal limb segment. The number of apodemes (points of insertion) per functional muscle, as well as the number of bundles attached to them, are variable according to the particular muscle group. All muscle bundles which move the coxopodite have their origin within the thorax. Similarly, five bundles (two depressor and three levator) controlling the basipodite have thoracic origins, thereby traversing both the C-B and T-C joints.

The remotor muscle consists of two seemingly discrete bundles which insert side by side on separate apodemes on the posterodorsal margin of the coxopodite. The posterior remotor bundle (1) originates proximally on the sternite, while the anterior remotor bundle (2) is attached to the interopleurite, the dorsoanterior wall adjoining the preceding thoracic segment.

The promotor muscle is comprised of two large bundles which have a common insertion on the anteroventral border of the coxopodite. The posterior promotor bundle (13) originates proximally on the sternite, while the anterior promotor bundle (2) is attached to the interosternite, the dorsoanterior wall adjoining the preceding thoracic segment.
Fig. 3. Dorsal in situ view of the main nerve tracts in the thoracic segment of the fifth pereiopod. The skeleton overlying the muscles has been removed and some of the dorsal muscle bundles laid back to expose the P5 nerve roots running into the base of the limb after their emergence from the thoracic ganglion. Muscle bundles shown are depressor (DEP), anterior levator (A. LEV), promotor (PRO), and the remotor (anterior remotor, A. REM.; posterior remotor, P. REM.), the individual bundles being numbered according to Figure 2. A. DIST. RT, anterior distal root; A. Th-Cx RT, anterior thoracicocoxal root; P. DIST. RT, posterior distal root; P. Th-Cx RT, posterior thoracicocoxal root.

The depressor muscle is composed of four bundles inserting on a single large apodeme that attaches to the ventral border of the basipodite. The two main depressor bundles (3, 4) extend beyond the coxopodite and originate in close association within the thorax. Bundle 3 originates on the medial interosternite and bundle 4 on the posterior sternite. The two remaining depressor bundles (5, 6) lie wholly within the coxopodite, originating on the internal surface of the ventral and posterodorsal cuticle.

The levator muscle complex appears to be divided anatomically into two distinct muscles—the anterior and posterior levators (see also Discussion). The large anterior levator muscle consists of four muscle bundles (7–10) inserting onto a single large apodeme on the anterodorsal rim of the basipodite. Three of these bundles (7–9) pass through the coxopodite and have their origins in the thorax. The most ventral bundle (7) originates from the medial interosternite; bundle 8 attaches to the posterior third of the interosternite, while the larger, more dorsal bundle 9 originates from the median structure. The remaining small anterior levator bundle (10) originates on the dorsal cuticle of the coxopodite and thus acts in a plane approximately perpendicular to that of the other three bundles. The posterior levator muscle is located entirely within the coxopodite and comprises two muscle bundles (11, 12) which originate on the dorsal coxal cuticle and insert onto a common apodeme immediately posterior to that of the anterior levator but at an angle of at least 40° to the latter’s main axis of movement. Whereas the function of the anterior levator muscle is clearly to act upon the C-B joint and move the limb anterodorsally, the precise role of the very much smaller posterior levator remains unclear (McVean, ’74; Moffett, ’75).

Thus, although the bicondylar articulation of each basal joint allows movement in a single plane only, the musculature controlling these joints is complex. In contrast to the distal leg segments (Wiersma, ’61), each of the four main basal muscles is subdivided into two or more distinct mus-
Fig. 4. Isolated nervous system of the thorax and coxa of the right fifth pereiopod. The anterior thoracicocoxal root (A. Th-Cx RT) innervates the anterior levator (A. LEV. v., ventral branch; A. LEV. d., dorsal branch) and promotor (PRO) muscles. It also carries sensory axons from the thoracicocoxal muscle receptor organ (TCMRO), the chordotonal receptor (CB) via the chordotonal nerve (CB NV), various epidermal receptors via the dorsal (D) and ventral (V) epidermal nerves (EPID. NV.), and the accessory nerve (ACC. NV.). The posterior thoracicocoxal root (P. Th-Cx RT) is purely motor and innervates the genital tract (G.T. c.), the depressor (DEP. d., dor-
sal branch), the remotor (P. REM. v., ventral branch; P. REM. d., dorsal branch; A. REM.), and the posterior levator (P. LEV.) muscles. The two Th-
Cx roots are connected by an anastomosis (ANAS) while the remaining P5 nerve roots (A. DIST. RT; P. DIST. RT) run together through the thorax
and coxa to the more distal limb segments. The A. DIST. RT also sends a ventral motor branch (DEP. v.) to the depressor muscle while the P. DIST.
RT carries axons from the cuticular stress detector (CSD1) located on the
proximal rim of the basi-ischiopodite. I-V indicate the location of cell body
clusters illustrated in Figures 5 and 6.

Thoracicocoxal nervous system

Peripheral innervation. In Carcinus the innervation to each pereiopod is carried in a cluster of nerve roots arising from the lateral margin of the thoracic ganglion (Fig. 3). Two of these, an anterior and a posterior root, arise more dorsally from the ganglionic neuropile to innervate the

mucles and sense organs of the thorax and coxopodite. The remaining nerve roots supply the basipodite and all the distal segments of the limb (see Silvey, '81). The distribution of axons and their major nerve branches within the two basal segments of P5 were determined by methylene blue staining and, in some cases, intracellular migration of cobaltous chloride.

Anterior thoracicocoxal root (A. Th-Cx RT). After emerging from the ganglion (Figs. 3, 4) this nerve root runs out in the thoracic segment of P5 between levator muscle

...
bundle 9 and promotor bundles 13 and 14 and terminates distally on the internal surface of the coxopodite. Functionally and anatomically the anterior Th-Cx nerve can be divided into two main parts: a proximal part which carries both motor and sensory axons, and a distal portion which is purely sensory. Proximally there are five major branches: (1) a sensory branch that innervates the thoraciccoxal muscle receptor organ (TCMRO) and the depressor muscle receptor (Alexandrowicz and Whitare, '57); (2) a mixed branch innervating the receptor muscle of TCMRO, the promotor (PRO) muscle, and the levator muscle receptor; (3) a dorsal motor branch (A. LEV. d.) carrying axons to bundle 9 of the anterior levator muscle; (4) a separate ventral motor branch (A. LEV. v.) to anterior levator bundles 7 and 8; and finally (5) a small branch (ANAS) that projects posteriorly to form an anastomosis with the posterior thoraciccoxal nerve root (see later).

The distal part of the anterior Th-Cx nerve was previously named the "chordotal nerve" since it carries sensory axons arising from the chordotonal receptor organ located at the coxo-basipodite (C-B) joint (Alexandrowicz, '67). However, we have found three additional distal branches of the A Th-Cx Rt which hitherto have been undescribed. The most proximal of these gives rise to what we have termed the "accessory nerve" (ACC NV.). This branch projects diagonally toward the distal limb nerve roots with which it runs in close apposition near the ventral surface of the coxopodite until the autotome plane (Fig. 4). Two secondary branches emerge from the midsection of the accessory nerve. One of these terminates in the connective tissue membrane separating the promotor and anterior levator muscles, while the other innervates the ventral epidermis of the coxopodite.

The anterior Th-Cx root gives off two further branches in the distal region of the thoracic segment. These are the dorsal and ventral "epidermal" (EPID.) nerves (Fig. 4). The former (D. EPID. NV.) projects to the membrane surrounding the apodeme of the remotor muscles, and the latter (V. EPID. NV.) innervates the levator apodeme membrane as well as the anterior epidermal surfaces of the coxopodite and perhaps the external coxal hairs. The remaining distal branch of the anterior root thus constitutes the chordotal nerve (CB NV.) sensu stricto that terminates in the CB chordotonal organ.

Two different populations of sensory cell bodies have so far been described in the TC/CB region of the crab walking limb (see locations I, V in Fig. 4). These are the peripheral somata of CB chordotonal receptor neurons (Fig. 5a; Alexandrowicz and Whitare, '57) and of cells belonging to the cuticular stress detector organ (CSD) whose axons are carried in a branch of one of the distal limb roots (Clarac, '76). In the present study, however, peripheral cell bodies have been found at three further locations (II, III, IV in Fig. 4) within the anterior Th-Cx root itself. The first location (II, Fig. 4) is in the dorsal epidermal nerve branch where one large bipolar cell (60 x 130 pm) has consistently been revealed with methylene blue staining. Another cluster of approximately ten bipolar cells lies in the peripheral part of the accessory nerve at the point where it runs in close apposition to the distal leg roots (IV, Figs. 4, 5b-d). The somata range in size from 30 to 50 pm x 70 to 125 pm and their dendritic processes are characteristically thicker near the soma boundary than the individual axon processes projecting back toward the ganglion (Fig. 5d).

In contrast to these distally located cells, there is a third group of about 15 bipolar and tripolar neurones which lies more proximally in the vicinity of the bifurcation between the main trunk of the anterior Th-Cx root and the accessory nerve (III, Fig. 4). These cells appear to correspond to the cluster of "nerve cells of unknown nature" reported by Alexandrowicz ('57) in the leg nerves of Palinurus. Cobalt or methylene blue staining (Fig. 6) reveal somata 30-40 pm x 40-80 pm in diameter and dendritic processes that run distally in either nerve branch (bipolar cells; Fig. 6a,b) or in both branches (tripolar cells; Fig. 6c,d). The cell bodies belonging to this group appear therefore to be located at some considerable distance from their peripheral terminals, although the precise destinations of the latter are currently unknown. Furthermore, the fact that tripolar neurones have dendrites projecting in both the accessory nerve branch and the distal part of the Th-Cx root suggests that if their function is sensory, as is likely, then they are involved in the processing of information from substantial and possibly multiple receptor fields. A possible functional clue is that the isolated soma location and morphology of these cells is strikingly similar to the multineurones of cutaneous mechanoreceptors in the abdominal segments of crayfish (Pabst and Kennedy, '67). Posterior thoraciccoxal root (P Th-Cx RT). This, the second of the two Th-Cx nerve roots, appears to be purely motor in function and runs distally from the thoracic ganglion between bundles 3 and 4 of the depressor muscle and of the posterior remotor bundle until it enters the coxopodite (Fig. 3). Proximally, this root gives rise to a thin branch which innervates the genital tract (G.T.) in male crabs before dividing into two main trunks (Fig. 4): a dorsal depressor branch (DEP d.) which carries motoneurones to the thoracic and coxopodite bundles of the depressor muscle, and a mixed levator-remotor branch. The latter innervates the posterior remotor muscle bundle via two separate dorsal and ventral branches (P. REM. d. and P. REM. v.), the anterior remotor bundle by a further branch (A. REM.), and more distally, the posterior levator muscle (P. LEV. v.). Also arising from this second major trunk is the anastomosis (ANAS) between the two thoraciccoxal roots.

There exists a further basal motor branch which is not derived from either of the main Th-Cx roots. The axons in this small nerve (DEP v; Fig. 4) emerge from the ganglion in the anterior distal root but diverge soon after to innervate the two thoracic bundles of the depressor muscle.

Central nervous organization of Th-Cx neurones. In brachyuran decapods, the ganglia of each thoracic and abdominal segment are compressed into a single enmeshed tissue mass, the thoracic ganglion, which is situated ventrally in the main cavity of the thorax (Fig. 1). The thoracic ganglion is connected anteriorly to the brain by the circumoesophageal connectives, and posteriorly to the abdomen via a bundle of abdominal nerve roots. In suitably illuminated preparations of unstained ganglia, it is possible to distinguish the individual segmental neuropiles from which arise the nerve roots supplying each of the five pairs of pereiopods. The following description is of the anatomy and cellular organization of the ganglionic neuropile corresponding to right P5.

Central projections of basal limb motoneurones. Cobalt backfills of axons in peripheral motor branches to the four main functional groups of basal limb muscles have revealed a spatial segregation of functionally related mo-
Fig. 5. Cobalt (a) and methylene blue (b–d) staining of basal limb sensory neurones. Bipolar somata located near their dendritic terminals (a) in the CB chordotonal organ (location I in Fig. 4), and (b–d) in the distal region of the accessory nerve (location IV). The inset (c) is a transverse section of the latter. Proximal direction is toward top (a) and left (b,d). Scale = a,b, 100 μm; c,d, 50 μm.

Fig. 6. Bipolar (a,b) and tripolar (c,d) somata located near the bifurcation of the accessory nerve from the main anterior thoracicocoxal root (location III in Fig. 4). Arrows indicate the direction of migration of cobalt ions in a–c. d shows a methylene blue-stained tripolar soma while e is a transverse section showing a single soma in the accessory nerve just after the point of bifurcation. In a–d, the direction of the ganglion is to the left. Scale = 100 μm.
toneurone somata within the ganglionic neuropile. The central projections of remotor/promotor and depressor/levator motoneurones are shown in Figure 7a, b, and c, d, respectively. The location of motoneurone somata remains remarkably similar both in bilateral backfills of the same ganglion, and from preparation to preparation. With one or two exceptions, the cell bodies of basal motoneurones lie clustered in the dorsal cortex of the ganglion. The majority of those motoneurones whose axons emerge in the anterior Th-Cx root (i.e., promotors, Fig. 7b; levators, Fig. 7d) have their cell bodies located in the anterior part of the P5 neuropile, while somata of motoneurones carried in the posterior Th-Cx root (i.e., remotors, Fig. 7a; depressors, Fig. 7c) lie mainly in the posterior region of the neuropile. Thus the somata of motoneurones innervating the two antagonistic pairs of basal limb muscles are organized correspondingly into two separate populations on opposite sides of the main axis of the P5 nerve roots (Fig. 8). Furthermore, there appears to be some spatial separation related to function within these two main soma populations. In the anterior group, promotor cell bodies tend to lie more medially than levator somata, while in the posterior group, the depressor cell bodies are concentrated near the midline of the ganglion while the remotors form a lateral cluster closer to the emergence of the posterior Th-Cx root (Fig. 8). The cell bodies range in diameter from 20 to 80 μm and appear to be devoid of any synaptic contacts (Figs. 7, 9). Each soma is connected by a single fine neurite (up to 800 μm in length) to a wide neuropilar process from which all the dendritic branches emerge. This broad central region has been termed the "integrating segment" by Sandeman ('69) for motoneurones in the brain of Carcinus maenas, and it is a feature of insect ganglia also (Burrows and Hoyle, '72). The integrating segment tapers distally into the initial segment of the axon, which then expands to form the main axon as it emerges from the neuropile in either of the two basal nerve roots.

A notable morphological property of thoracicocoxal motoneurones is the close contiguity of their neuropilar processes. This is most evident for cells innervating muscles...
belonging to the same functional group. In Figure 9, for example, the central projections of remotor and depressor motoneurones display quite similar and parallel branching patterns within each population. The contiguity extends from the soma neurites at the origin of the integrating segments to where the latter form the axons at the lateral margin of the neuropile. Furthermore, combination staining of different functional motoneurones has shown that the initial axon segments contributing to each group do not arise separately from different regions of the neuropile. Rather, the integrating segments and major dendrites of the entire motoneurone population overlap each other closely, contributing equally to a single dense neuropilar plexus. The motoneurone dendrites also appear to be strictly segmental in the extent of their distribution since no neurites have been seen to cross the midline of the ganglion to the contralateral P5 neuropile, or to invade the ipsilateral neuropile of the preceding (P4) thoracic segment.

Backfills of the same peripheral nerve branches in different preparations have also indicated the number of motoneurones innervating each of the four main basal muscles. In well-stained ganglia, it is generally possible to count the somata of seven promotor motoneurones, seven remotors, eight anterior levators, and ten depressors. However, these estimates derived from separate backfills of each group ignore the possibility of common innervation to different functional muscles. Thus of the total of 30 or so stained somata, one or more cell body in different groups might belong to the same motoneurone. That this can be the case is illustrated by the preparation shown in Figure 10. This particular backfill from the depressor nerve branch of the posterior Th-Cx root after the latter had been cut close to the ganglion revealed a single soma whose axon emerges from the ganglion in the anterior root and then branches extensively and runs via the anastomosis to innervate all thoracicocoxal muscles, as well as sending a process in a nerve root to the more distal limb segments. It is interesting to note, furthermore, that the soma location of this common motoneurone near the midline between left and right P5 neuropiles corresponds to the position of the isolated posterior cell body consistently observed in separate backfills of the promotor and anterior levator motor branches (cf. Fig. 10 with Fig. 7b,d, respectively). Further speculation on the basis of the innervation by this cell of several different muscles suggests that it may be a common inhibitory motoneurone, as described originally by Wiersma ('61) for the distal leg muscles of decapod crustaceans.

Neuropilar projections of coxopodite sensory neurones. As for most crustacean mechanoreceptor neurones (Mill, '76) the cell bodies of the chordotonal organ of the coxopodite are located peripherally near their terminals in the receptor complex itself (Fig. 5). We have also used orthograde migration of cobalt to trace the central terminals of these sensory elements within the thoracic ganglion (Fig. 11). The chordotonal axons enter the ganglion via the anterior Th-Cx root and immediately taper into a bundle of thin processes which curve posteriorly with little arborization to become lost in the dorsal neuropile (Fig. 11a). The axis of these afferent projections is the same as that invaded by the integrating segments and dendrites of the basal motoneurones (Fig. 11b), thereby providing an appropriate geometry for the possible formation of direct reflex path-
CRAB BASAL LIMB NEUROMUSCULAR ORGANIZATION

Fig. 10. Photographic montage and diagram showing common innervation of basal limb muscles by a single motoneurone. In this preparation (dorsal view) the post. Th-Cx nerve root was cut near its emergence from the ganglion so that the only anatomical connection between the two was via the anastomosis and the ant. Th-Cx root. Subsequent cobalt backfilling of the dorsal depressor branch (arrow) of the post. Th-Cx root revealed a motoneurone which sends additional axon branches to the remotor and posterior levator muscles, then crosses in the anastomosis to innervate the levator and promotor muscles before entering the ganglion via the ant. Th-Cx root. A further branch projects in the ventral depressor nerve and to the distal limb segments in the anterior distal root. Note the position of the cell's soma near the posterior midline of the ganglion. Scale = 200 µm.

ways between the two. A similar close association between chordotonal organ afferents and motoneurone dendrites occurs in the chelipeds (first pereiopods) of crayfish (Wiens, '76) and here the use of spike train analysis (Wiens and Gerstein, '76) has also demonstrated the likely existence of monosynaptic connections.

DISCUSSION

Functional correlates of basal limb anatomy

One of the main purposes of this paper is to provide a general anatomical description of the skeletonmusculature and innervation pattern of the two proximal segments (thorax and coxopodite) of the shore crab fifth pereiopod. This region plays an important role in the behavioural activity of the limb as a whole; the promotor/remotor muscles operating the T-C joint maintain the spatial orientation of the leg, while those controlling the C-B joint (levator/depressor muscles) appear to be involved in both postural and locomotory behaviour (Clarac and Coulmance, '71; White and Spirito, '73, Bévengut, '82). Although these individual muscles are subdivided into more or less distinct groups of fibre bundles, the anatomy within each group is complex and in some cases raises doubts as to whether apparently related muscle bundles comprise a single functional unit. One anatomical correlate of a functional muscle is the insertion of constituent fiber bundles onto a common apodeme, thereby imparting the same direction of movement about the joint they control. On this basis, therefore, the groups of basal promotor and depressor muscle bundles can be defined as single entities. In contrast, however, the two remotor bundles of P5 have separate insertions and thus could be considered as distinct muscles, as suggested by McVean ('73) for Carcinus, and White and Spirito ('73) for the swimming crab Callinectes. However, further considerations indicate that, for Carcinus at least, this is not the case. First, fibres of both remotor bundles act strictly in parallel since they overlie each other and have similar origins. Second, their contraction can cause movement in a single plane only, due to the skeletal arrangement of the T-C joint. Third, the two remotor bundles tend to operate synergistically during behaviour,
since much of their excitatory innervation is shared between the same motoneurones (Simmers and Bevengut, in preparation). This has been further confirmed by simultaneous EMG recordings from the remotor bundles during locomotory behaviour in the intact animal (Clarac, unpublished observations).

The need to correlate anatomy with function also applies to the basal levator musculature, whose various bundles are responsible for moving the leg in a dorsorostral direction about the single axis of the C-B joint. Several lines of evidence, however, suggest that there are two distinct levator muscles—an anterior and a posterior unit (see also McVean, '73; White and Spirito, '73). The three main anterior levator bundles all originate in the thorax, are attached to a common tendon, and share the same motoneurones. In contrast, the two smaller posterior levator bundles have a completely different disposition, being located entirely within the coxopodite. They also have a separate tendon and largely different motor innervation to that of the anterior levator group. These two groups of levator bundles are thus capable of operating independently and this property, together with their structural differences, may underlie separate functions such as "phasic" and "tonic" control of limb movement or a specialised role of the posterior levator muscle in limb autotomy (McVean, '74; but cf. Moffett, '75).

The musculature of the basal region of the crab walking leg exhibits a degree of structural complexity that is considerably greater than that of other crustacean limb muscles, including those in the more distal segments of the same appendage (Wiersma, '61). Whereas each basal muscle consists of at least two more or less discrete bundles, the distal leg segments contain one pair of antagonistic muscles that are seldom divided into bundles. Furthermore, the muscles of the thorax and coxopodite receive a total of at least 30 independent motoneurones. In contrast, the eight remaining distal leg muscles are innervated by a total of only 15-17 motoneurones (Wiersma, '61; Silvey, '81). Anatomical differences such as these may in turn reflect the relative importance of the role played by the basal neuromuscular system in postural and locomotory behaviour.

The present study also permits comparison between the shore crab and another brachyuran species, the blue crab Curcinus, whose fifth pereiopods are used specifically for swimming locomotor activity (White and Spirito, '73). Although the back legs of Curcinus are considered as "walking appendages," their function appears to be different from the other pairs of thoracic limbs, being more to support the body during locomotion than to provide active propulsion (Clarac and Coulmande, '71). However, when the animal is lifted above the substrate, this pair of legs displays rotational sculling movements and appropriate muscle activity which are very similar to those of the P5e of "true" swimming crabs (Clarac, unpublished observations). Further observations on unrestrained Curcinus also suggest that swimming-type locomotion involving only the back legs, although it occurs infrequently, is part of the normal behavioural repertoire of the animal.

The fifth pereiopods of Curcinus and Callinectes also display significant morphological similarities. In both cases the distal segments of P5 have become flattened into an oarlike structure, although this is considerably more pronounced in Callinectes, where the dactyl, in particular, is expanded to form a large blade. A further major external similarity is that in both species the back legs show a 90° rotation of the T-C and C-B joints in relation to the remaining walking limbs. It is the alteration in limb insertion that redirects the main propulsive forces exerted about the C-B joint from a near vertical plane (as required for walking), to a plane along the horizontal axis for swimming (White and Spirito, '73). Associated with this change, furthermore, is a flattening of the posterior part of the thorax, especially in Callinectes, so that during swimming the more distal leg segments are able to swing in a greater arc about the coxopodite and over the back. Internally, the basal portion of the fifth pereiopod in the two crabs also appears to be similar in terms of general organization and structural complexity, although compared to the other walking limbs, the muscles of P5 in Callinectes are considerably larger and more powerful than in Curcinus. This probably underlies the relative occurrence of walking- and swimming-type locomotion in the two animals. The intertidal habitat of Curcinus may shed some light on the apparent subsidiary role that swimming plays in this species. Nonetheless, the fact...
that the shore crab possesses both the neural and muscular machinery for swimming places it relatively close to highly adapted species such as Callinectes in the evolution of this type of locomotory behaviour.

Central nervous organization

The central projections of basal limb motoneurones have morphological features typical of arthropods in general (for reviews see: Evoy, '77; Fourtner and Pearson, '77). All cell bodies are unipolar and located some distance from the main neuropilar segment, each of which gives rise to a network of dendritic branches before tapering into the initial axon segment at the lateral margin of the ganglion. The enlarged central processes of basal motoneurones are probably the integrating regions where synaptic currents arising in the dendrites of individual cells are summed and conveyed to the impulse initiating zone located at some point along the initial axon segment (Sandeman, '69; Burrows and Hoyle, '72).

A striking feature of basal motoneuronal geometry is the close structural relationship between their major neuropilar processes and dendritic fields. This is most noticeable for functionally related motoneurones, whose neurites overlap each other closely, displaying coincident branching patterns and parallel dendritic structures. Moreover, there exists a close contiguity between the main neurites and dendrites of different functional groups of motoneurones. Whereas the somata of each group tend to lie in different regions of the ganglion, the integrating segments of the entire population of basal motoneurones run together in a single large tract, and their finer processes intermingle and ramify in the same region of the neuropile. It is interesting to note, furthermore, that this dendritic plexus encircles and invades the axis of the P5 nerve roots along which proprioceptive afferents, such as those arising from the CB chordotonal organ, enter the ganglion. Such an overlapping topography seems appropriate for two possible functional aspects of the central nervous organization of the thoracic limb system; namely, the sharing of synaptic inputs from the same presynaptic sensory or interneuronal source, and second, the possibility of direct collateral interactions between the motoneurones themselves. Both these mechanisms have been shown to play important integrative roles in the neural circuitry of crustacean motor systems, in particular (e.g., Mulloney and Selverston, '74; Wiens and Gerstein, '76; Wiens and Atwood, '78; Heitler, '78), and electrophysiological evidence for their existence, in addition to that suggested by anatomy, is currently being sought in the crab locomotory system.

One remaining feature of the neuropilar distribution of basal motoneurones is that their somata and dendritic fields appear to be confined entirely to the ipsilateral side of the ganglion. There are therefore no sites, so far as we can determine from our intensified cobalt-stained preparations, at which direct coupling between contralateral motoneuronal collaterals could occur. Observations on other arthropod motor systems have indicated a general relationship between the crossing over and intermingling of bilaterally homologous motor units and the degree of synchronization between the appendages they control. For example, movement of the crayfish abdomen is bilaterally symmetrical and correspondingly, many abdominal motoneurones have extensive arborisations in contralateral hemimagelia (Selverston and Remler, '72; Wine et al., '74). Conversely, in bilaterally uncoupled motor systems, such as the reflex behaviour of the crayfish claw (Wiens, '76) and eyestalk movements in crabs (Sandeman and Okajima, '73), the motoneurones are located wholly within the neuropile of the hemimagelia from which the axons emerge. On this basis, therefore, left and right pairs of legs in crabs might be expected to operate independently during locomotory behaviour. The observation that contralateral limbs are relatively tightly coupled, albeit asynchronously (Clarac and Coulmann, '71; Seinis and Silvey, '80), suggests that here bilateral coordination is mediated by interneuronal pathways. Transganglionic interneurones appear also to be responsible for the bilateral reflex activation of lobster claw motoneurones which have ipsilateral dendritic domains but, unlike the crayfish, are excited by contralateral sensory input (Govind et al., '79).

ACKNOWLEDGMENTS

We wish to thank Drs. B.M.H. Bush and B.G. Horseman for helpful discussions and for critically reading early drafts of the manuscript. This work was supported by grants from C.N.R.S. (ATP 82), D.G.R.S.T. (80 P 6049), F.R.M.F., and the French Foreign Ministry.

LITERATURE CITED


