

Functional organisation of anterior thoracic stretch receptors in the deep-sea isopod *Bathynomus doederleini*: behavioural, morphological and physiological studies

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Summary

The relationship between segmental mobility and the organisation of thoracic stretch receptors was examined in the deep-sea isopod *Bathynomus doederleini*, which shows a developed adaptive behaviour during digging. The movements of segments during digging were analysed from video recordings, which showed that a large excursion occurred in the anterior thoracic segments. Dye-fills of axons revealed four types of thoracic stretch receptor (TSR): an N-cell type (TSR-1), a differentiated N-cell type (TSR-2), a muscle receptor organ (MRO)-type with a long, single receptor muscle (TSR-3) and an MRO-type with a short, single receptor muscle (TSR-4 to TSR-7).

Physiologically, TSR-1 and TSR-2 are tonic-type stretch receptors. TSR-3 to TSR-7 show two kinds of stretch-activated responses, a tonic response and a phasico-tonic

response in which responses are maintained as long as the stretch stimulus is delivered. Both TSR-2, with a long muscle strand, and TSR-3, with a single, long receptor muscle, have a wide dynamic range in their stretch-activated response. In addition, TSR-2 is controlled by an intersegmental inhibitory reflex from TSR-3. These results suggest that, although TSR-1 has no receptor muscle and TSR-2 has a less-differentiated receptor-like muscle, they are fully functional position detectors of segmental movements, as are the MRO-type receptors TSR-3 to TSR-7.

Key words: stretch receptor, muscle receptor organ, N-cell, accessory neurone, crayfish, Crustacea, isopod, *Bathynomus doederleini*.

Introduction

The presence of muscle receptor organs (MROs) in the posterior thorax and the abdomen was first reported in *Homarus vulgaris* and *Palinurus vulgaris* by Alexandrowicz (Alexandrowicz, 1951; Alexandrowicz, 1952). Since then, MROs have been found in a wide variety of decapods, isopods, mysids and stomatopods (Alexandrowicz, 1954; Alexandrowicz, 1956; Pilgrim, 1960; Pilgrim, 1964; Bush and Laverack, 1982; Rydqvist, 1992; Wallis et al., 1995). In crayfish, the abdominal MRO consists of a pair of receptor cells with receptor muscles, together with motor innervation of the receptor muscles and inhibitory innervation of the receptor cells. It is thought to play a role in controlling abdominal posture (Fields, 1966; Sokolove, 1973; McCarthy and Macmillan, 1995). In the anterior thorax of *H. vulgaris* and *P. vulgaris*, there are stretch receptors without differentiated receptor muscles. Alexandrowicz (Alexandrowicz, 1952) referred to these stretch receptors as N-cells and suggested that N-cells might represent a primitive form of MRO. Alternatively, Alexandrowicz (Alexandrowicz, 1956) proposed, in a study of receptor elements of *Leander serratus*, that 'the N-cell might be the remnants of the retrograding receptor organs, presumably from MRO1'.

It has been argued, from decapod examples, that N-cells may be associated with reductions or changes in segmental mobility. This hypothesis was supported by studies on thoracic stretch receptors of the crayfish and rock lobster (Wiersma and Pilgrim, 1961) and of the hermit crab (Pilgrim, 1974). Pilgrim (Pilgrim, 1964) also reported that in the more primitive Malacostraca, which are stomatopods with free thoracic segments, fully developed MROs were present in the posterior four thoracic segments and that the most anterior thoracic segment had a stretch receptor that could be 'tentatively regarded' as an N-cell. Little is known about the function of the N-cell except that it responds to a stretch stimulus with slowly adapting impulse discharges (Wiersma and Pilgrim, 1961). However, Macmillan and Field (Macmillan and Field, 1994) advanced our understanding of the N-cell in the crayfish *Cherax destructor* by characterising the responses of the N-cell to active contraction of the muscle on which the dendrites of the N-cell terminate. Contractions were evoked by electrical stimulation and passive muscle stretching. As a consequence of this work, they proposed that N-cells monitor postural and locomotory movements in the less mobile thorax.

As mentioned above, the relationship between segmental mobility and the morphology of stretch receptors has been examined in crustaceans with a carapace. Crustaceans such as isopods and the primitive syncarid *Anaspides tasmaniae*, which are articulated along all, or almost all, of their entire body, should provide another opportunity to study N-cells, if they are present in the anterior thorax (Wallis et al., 1995). Along these lines, we have previously examined the isopods *Armadillidium vulgare* and *Ligia exotica* (Niida et al., 1990; Niida et al., 1995b; Niida et al., 1998). In *A. vulgare*, which is noted for its rolling-up and digging behaviour, all the paired thoracic stretch receptors are of the slowly adapting type. In *L. exotica*, which is characterized by its fast locomotor activity and for its failure to roll up, paired stretch receptors are of the rapidly and slowly adapting types, except in the second thoracic segment. Morphologically, the anterior thoracic stretch receptors of these isopods are similar to the N-cells of decapods and the posterior thoracic stretch receptors are similar to the MRO-like stretch receptors.

These on-going studies have yet to determine whether the MRO/N-cell distinction in thoracic stretch receptors is related to joint mobility. We have also examined the thoracic stretch receptors of the deep-sea isopod *Bathynomus doederleini*, whose stretch receptors may reflect the development of adaptive behaviour, since this animal has a more distinctive pattern of digging behaviour than that of *A. vulgare* (Sekiguchi, 1985).

In this report, we first describe the movements of the anterior thoracic segments during burrowing in *B. doederleini* and then identify the morphological and physiological characteristics of the most anteriorly located thoracic stretch receptors and of the neighbouring stretch receptors associated with segmental movements. Some of these findings have been reported previously in abstract form (Niida et al., 1995a).

Materials and methods

Animals

Male and female specimens of the deep-sea isopod *Bathynomus doederleini* (Ortmann, 1894) were collected in baited traps set on the sea floor at a depth of 200–500 m in the Sea of Kumano during cruises 93–99 of the training vessel *Seisui-maru* (Faculty of Bioresources, Mie University, Japan). These animals, which measured 4–14 cm in total length, were maintained in recirculated sea water at 14 °C on a diet of fresh crayfish.

Recording of behaviour

The digging behaviour of freely moving *B. doederleini* was recorded on VHS videotape with a Victor video camera, and the relevant data were viewed with a Victor colour monitor. Fig. 1A shows the apparatus used for these behavioural experiments. Different materials were tested for their suitability as a substitute for the natural substratum. Gelatine was chosen because its transparency makes it easy to observe digging behaviour and its firmness facilitated the formation of a burrow.

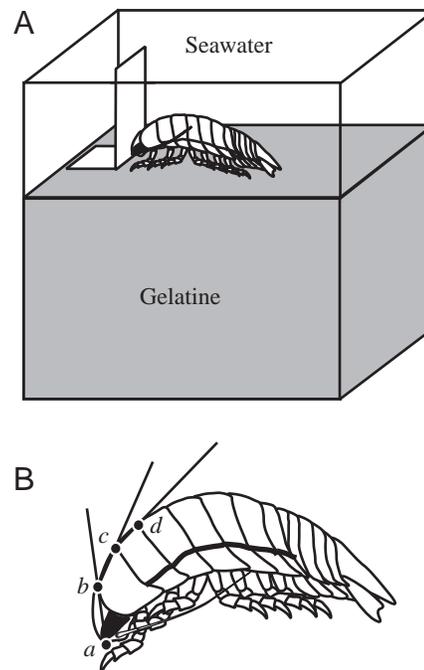


Fig. 1. Experimental arrangement for recording digging behaviour (A) and the method used to measure segmental deflection (B). (A) L-shaped acrylic walls were placed in an aquarium to induce digging behaviour. Solid gelatine served as a substratum. The sea water was aerated and kept at 14 °C. (B) Points *a* and *b* indicate the anterior and posterior margins of the head, respectively. Similarly, points *c* and *d* indicate the posterior margins of the second and third thoracic segments, respectively. For the second thoracic segment, lines *ab* and *bc* formed the angles of extension and flexion. For a more detailed explanation, see the text.

An aquarium containing gelatine (1.6% w/v in sea water) was used to simulate sea-bottom sediment. An L-shaped wall made of acrylic plastic was placed away from the lateral side of the aquarium (Fig. 1A). Whenever the animal made contact with the wall with its head, it began to make a burrow. In the absence of the L-shaped wall, the animal dug along the side of the aquarium, which impaired the observation of normal segmental flexion and extension. The angle of each thoracic segment under flexed and extended conditions was measured by placing a clear acetate sheet on the video monitor and tracing the segmental postures of flexed and extended animals.

The angles of extension and flexion of the thoracic segments were defined as the angles between two segments; for example, as seen in Fig. 1B, the angle of the second thoracic segment is formed by line *ab*, which is drawn from the front of the head to the posterior edge of the head, and line *bc*, drawn from the anterior to the posterior edge of the second thoracic segment. Similarly, the angle of the third thoracic segment is formed by lines *bc* and *cd*. The terga of successive segments are arranged in a curve even when the animal is not digging. Thus, to determine absolute angles, each segmental angle was subtracted from the respective segmental angle in the animal at rest.

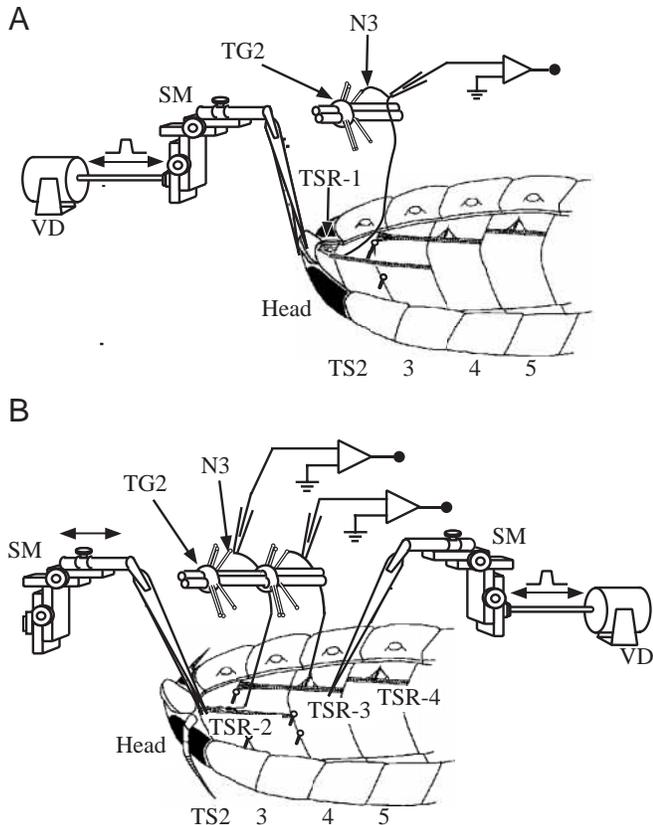


Fig. 2. Procedure (A) for recording stretch-activated responses of TSR-1 and (B) for demonstrating intersegmental inhibition of TSR-2 by TSR-3. All the ganglia except the first (A) and the second and the third (B) thoracic ganglia were cut off. TSR-1 was activated by moving the head in a horizontal direction with forceps mounted on a small micromanipulator connected to a vibration device. Stretch-activated responses of TSRs were recorded *en passant* from N3. N3, nerve 3; TG1, first thoracic ganglion; TS2, second thoracic segment; TSRs, thoracic stretch receptors; VD, vibration device; SM, small micromanipulator.

Morphology

To identify segmental thoracic stretch receptors, we used vital staining with Methylene Blue and axonal filling with Lucifer Yellow or nickel chloride (NiCl_2). The cut end of the peripheral stump of the third segmental nerve (N3) in each thoracic ganglion was introduced into a polyethylene tube filled with 5% (w/v in aqueous solution) Lucifer Yellow or 0.5 mol l^{-1} NiCl_2 . For axonal filling with Lucifer Yellow, negative current pulses of 0.6 A (0.5 s duration) were passed for 1.5 h through the polyethylene tube. For NiCl_2 filling, no current was applied. The NiCl_2 filling was carried out at 4°C for 12 h to allow for the diffusion of NiCl_2 , which was precipitated by the addition of rubeanic acid. Both Lucifer-Yellow- and NiCl_2 -filled preparations were then fixed in 10% formalin for 2 h , washed for 1 h , mounted in gelatine on a slide and clarified in glycerol or methyl salicylate. To reveal the central projections of the stretch receptor axons, the cut end of the central stump of the third nerve of each thoracic ganglion was introduced into a polyethylene tube filled with 5% Lucifer Yellow, and current

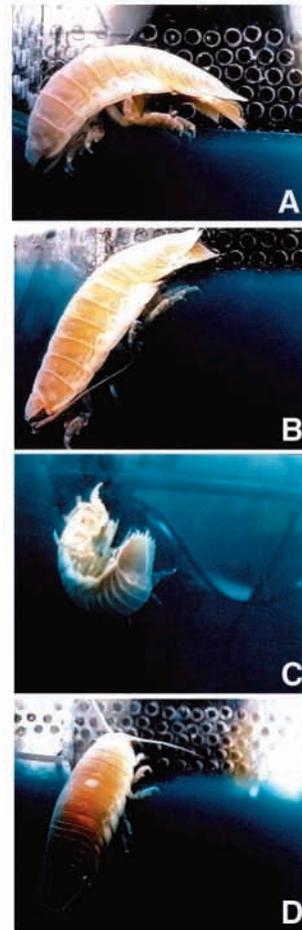


Fig. 3. Digging behaviour of *Bathynomus doederleini*. Video frames show (A) the start of digging of a burrow, (B) advancing towards the bottom, using the thoracic legs and beating swimmerets, (C) reversing direction by rolling up to leave the burrow and (D) creeping out of the burrow. This animal was 11 cm long.

was passed as for the application of Lucifer Yellow towards the periphery.

Physiology

Preparations for recording electrical activity

After the animals had been anaesthetised in cold sea water, all the segments, except the head and the second, third and fourth thoracic segments, were cut off together with all the legs. The viscera were then dissected away from the cut end of the fifth thoracic segment, and the nerve cord behind the fifth thoracic segment was removed. To prevent the deterioration of stretch receptors and nerve tissue caused by endogenous digestive enzymes, these preparations were immediately flushed with physiological saline for *B. doederleini* (Tsukamoto et al., 2000): (mmol l^{-1}) Na^+ , 479.4 ; K^+ , 15.7 ; Ca^{2+} , 14.6 ; Mg^{2+} , 60.7 ; Cl^- , 627.6 ; SO_4^{2-} , 0.91 ; buffered to $\text{pH} 7.9$ with Hepes.

Stretch stimulus and recording of stretch-activated responses

Stretch stimuli for TSR-1 were produced by pulling the head

with forceps mounted on a small micromanipulator (C-2, Narishige) connected to the moving central pin of a vibration device. The vibration device was driven by applying a ramp-and-hold pulse (Fig. 2A). The muscle strand or receptor muscles of TSR-2, TSR-3 and TSR-4 were directly stretched with the forceps in the same way (Fig. 2B). Stretch-activated responses were recorded *en passant* through suction electrodes from N3 in the relevant ganglion (Fig. 2A,B). Inhibitory effects between thoracic stretch receptors were examined by attaching a second stretching device to the caudal thoracic stretch receptor of an adjacent pair. Fig. 2B illustrates the arrangement for stretching TSR-3 and recording from TSR-2.

Results

Digging behaviour

Sekiguchi (Sekiguchi, 1985) observed experimentally that, when freed onto deep-sea sediment, *B. doederleini* made a burrow in the substratum. For further qualitative analysis of segmental movement, we studied digging behaviour using gelatine as a substitute for sea-bottom sediment. When *B. doederleini* moves around on the gelatine substratum and its rostrum hits a wall of the aquarium, it begins to dig a burrow. Fig. 3 shows a sequence from the start of digging to creeping out of the burrow. First, the animal digs a burrow, using the first to third thoracic legs, simultaneously moving the debris backwards. The remaining legs are used to support the body and provide the driving force to move towards the bottom. The gelatine debris is moved away from the burrow with the help of the water currents produced by vigorous beating of the swimmerets, which at other times beat slowly and serve as respiratory organs. The movements of the thoracic legs are synchronised with the repeated extensions and flexions of the thoracic segments, so that the head and thorax are pushed into the gelatine substratum, making a burrow. The extent of the extension and flexion of the thoracic segments is shown in Fig. 4A, in which, since there is large variation in the angle of flexion and extension in each body segment, it is difficult to determine which body segment has the largest angular movement. However, it is safe to say that even an anterior segment, such as the second thoracic segment, is movable to a considerable extent. In this behavioural experiment, the angle between full extension and full flexion in the second thoracic segment was 30° . Like *A. vulgare*, *B. doederleini* also rolls up when its legs are lifted from the substratum, but its final shape is not as ball-like as that of *A. vulgare*. Fig. 4B shows that, when the animal is forced to roll up, the flexion is greatest around the fourth thoracic segment.

Such flexion occurs voluntarily during digging; since *B. doederleini* makes a burrow with only one entrance, the animal must reverse its direction by rolling up to exit the burrow (Fig. 3C). The animal then pauses at the burrow entrance before creeping out (Fig. 3D).

Overview of the nervous system of the thorax

For the anatomical nomenclature of the central nervous

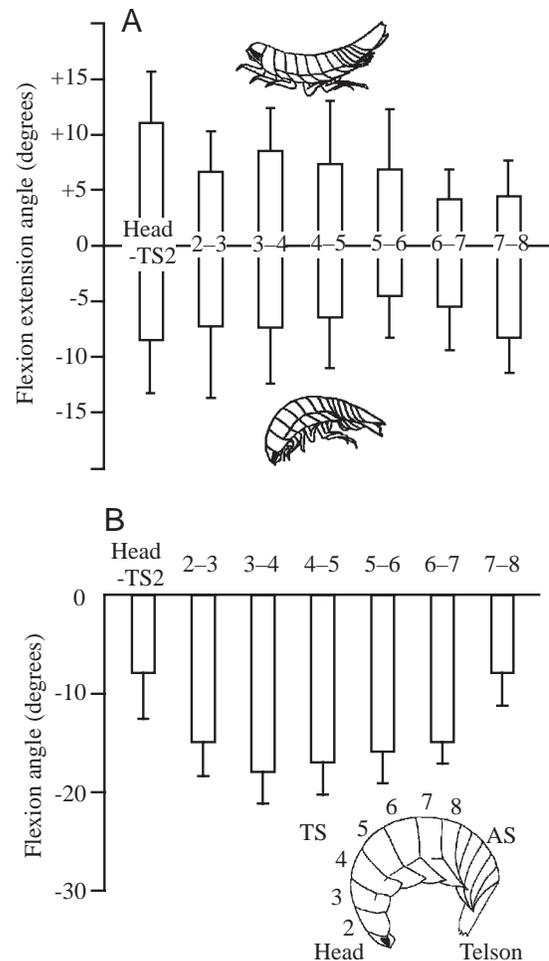


Fig. 4. (A) Flexion and extension of the thoracic and abdominal segments during digging. Measurements were based on 6–8 selected video frames per animal ($N=8$). The drawings show extension and flexion of body segments. (B) The maximum angles of body segments during roll-up. The position of the animal during rolling up is shown in the drawing. Measurements were made as in A. Each column in A and B represents the mean value \pm S.E.M. AS, abdominal segment; TS, thoracic segment. Numerals indicate numbers of body segments.

system (CNS) in isopods, we adopted that used by Walker (Walker, 1935). The thoracic segments contain eight thoracic ganglia, with the first thoracic ganglion (TG1 in Fig. 5) being smaller than the rest. This ganglion is also referred to as a maxillipedal ganglion (Walker, 1935; Thompson et al., 1994). Each ganglion provides three segmental nerves; the first, second and third nerves (N1, N2 and N3 in Fig. 5), of which N1 and N2 innervate the leg muscle (not shown) and N3 contains the axons of the thoracic stretch receptor and dorsal extensor and ventral flexor motor axons. The segmental nerves of the thoracic ganglia in this animal are briefly compared with those in crayfish below. According to Elson (Elson, 1996), the thoracic ganglia of crayfish, such as T4 (the fourth thoracic leg ganglion—the seventh thoracic neuromere), give off three segmental nerves: N1, N2 and N3. N1 consists of a cluster of

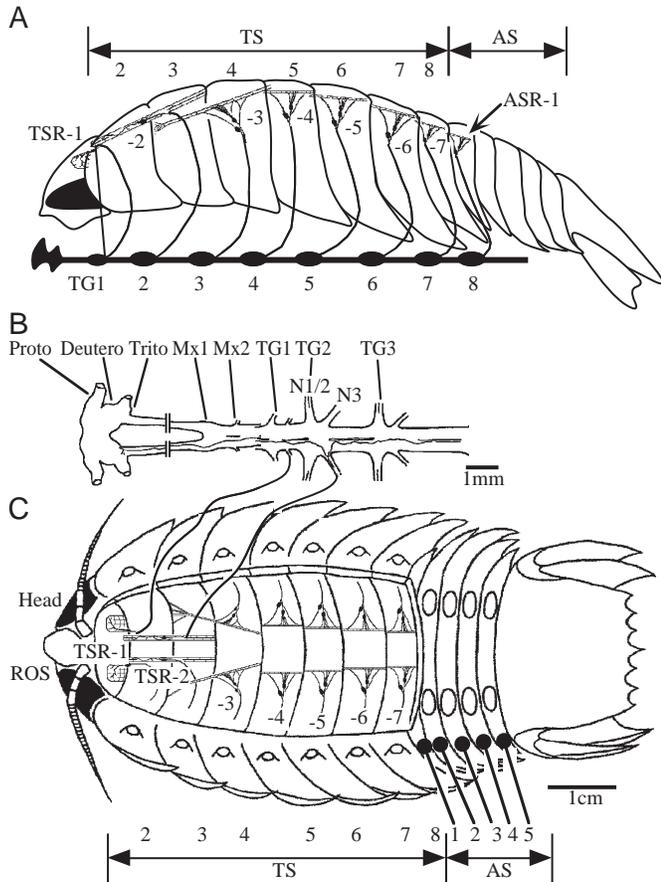


Fig. 5. (A) Lateral view of the spatial organisation of thoracic and abdominal stretch receptors. Segmental stretch receptors are located on the dorsal side and extend axons that run towards the central nervous system (CNS) *via* nerve 3 (N3). Note that, as in crayfish, the axons of the abdominal stretch receptors project to the eighth thoracic ganglion. (B) The CNS and central projection of the axon of TSR-2. The CNS is depicted dorsally, and the CNS posterior to TG3 is not drawn, and the oesophageal connectives are interrupted. Ascending and descending central projections of the axon of TSR-2 are shown (*camera lucida* drawing). This was revealed by electrophoretically applying Lucifer Yellow to the centrifugal cut end of N3. (C) Ventral view of the organisation of thoracic stretch receptors. Thoracic stretch receptors are located dorsally and bilaterally. Dendritic branches from the receptor cell of TSR-1 innervate the extensor muscle, while those of TSR-2 entwine with the receptor muscle-like strand, which is exaggerated in size. TSR-3 to TSR-7 have two receptor cells and a single receptor muscle. AS, abdominal segment; ASR, abdominal stretch receptor; TS, thoracic segment; TSR, thoracic stretch receptor; ROS, rostrum; Deutero, deutocerebrum; Mx, maxillary nerve; Proto, protocerebrum; Trito, tritocerebrum; TG, thoracic ganglion.

nerve, among which two large branches, N1AV and N1PV, run towards the leg muscle. Of the smaller branches, N2 innervates the extensor muscle, while N3 innervates the flexor muscle. Our unpublished data for crayfish (*Procambarus clarkii*) show that the axon of a thoracic stretch receptor projected to the CNS through N2. Consequently, N3 of the

thoracic ganglion in an isopod corresponds to N2+N3 of the thoracic ganglion in crayfish.

Morphology of thoracic stretch receptors

In this study, we renumbered the thoracic stretch receptors so that the nomenclature would correspond to that in our studies on isopods. Numerals following the designation TSR represent the number of the thoracic ganglion to which the third thoracic segmental nerve belongs (Fig. 5). The thoracic stretch receptors of *B. doederleini* are spatially organised as shown in Fig. 5, in which the first thoracic body segment is not depicted since, in Isopoda, it is considered to be fused with the head. The axon of a thoracic stretch receptor in a given thoracic segment projects to the CNS through the third segmental nerve (N3). To avoid confusion, the spatial arrangement of thoracic stretch receptors is described for one side only.

TSR-1

The cell body of TSR-1, which was successfully labelled with Lucifer Yellow in five specimens, has a long spindle shape and usually lies on the anterior ridge of the second thoracic segment, but occasionally on the extensor muscle within the head (Fig. 5). This extensor muscle spans the head and the anterior ridge of the second thoracic segment. To the fascia of the extensor muscle are attached many dendritic branches derived from the stout dendritic process of TSR-1. As shown in the Lucifer-Yellow-filled cell in Fig. 6, many dendritic branches of TSR-1 fan out on the extensor muscle, where we can also see innervation by one motor neurone. This runs laterally from the fusiform receptor cell of TSR-1. Thus, this stretch receptor has no differentiated receptor muscles. The axon from the receptor cell reached the connectives *via* N3 of the first thoracic ganglion, and then its axon, like that of TSR-2, bifurcated into a descending and an ascending axon (not shown).

TSR-2

This type of thoracic stretch receptor consists of a muscle strand and one receptor cell (Fig. 7), the soma of which is located in the second thoracic segment (Fig. 5). The muscle strand, composed of two muscle fibres (see area enclosed by dotted lines in Fig. 7A and asterisks in Fig. 7B,C), runs medially in close parallel with the deep extensor muscle, whose fibres are larger than those of TSR-2. The anterior insertion of the muscle strand of TSR-2 is on the anterior ridge of the second thoracic segment, and the posterior insertion of the muscle strand of TSR-2 is on the anterior ridge of the fourth thoracic segment (Fig. 5A,C). As shown in Fig. 7A-C, the fusiform receptor cell of TSR-2 is oriented longitudinally on the muscle strand. Among 11 labelled TSR-2s, there were some differences in morphology. In Fig. 7A, a pair of stout dendrites extend medially and anteriorly from a receptor cell body, whereas in Fig. 7B, lateral and anterior dendrites can be seen, and in Fig. 7C, there is only one anteriorly directed stem dendrite. It is not clear at present whether these morphological differences are due to morphological variation

Fig. 6. TSR-1 revealed by the application of Lucifer Yellow to the peripheral cut end of the dorsal nerve. Dendritic branches from the anterior pole of a fusiform receptor cell fan out on the extensor muscle, and a motor nerve (MN) runs antero-medially from the posterior pole. The inset shows the area around the motor nerve indicated by the arrow, demonstrating that fine branches extend out from the large MN. The MN was broken artificially. RC, receptor cell. Scale bar, 200 μ m.

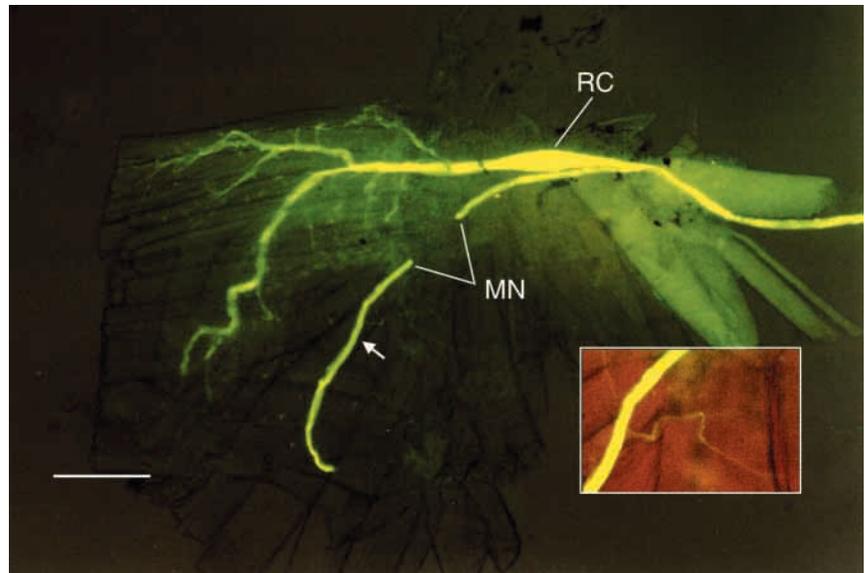
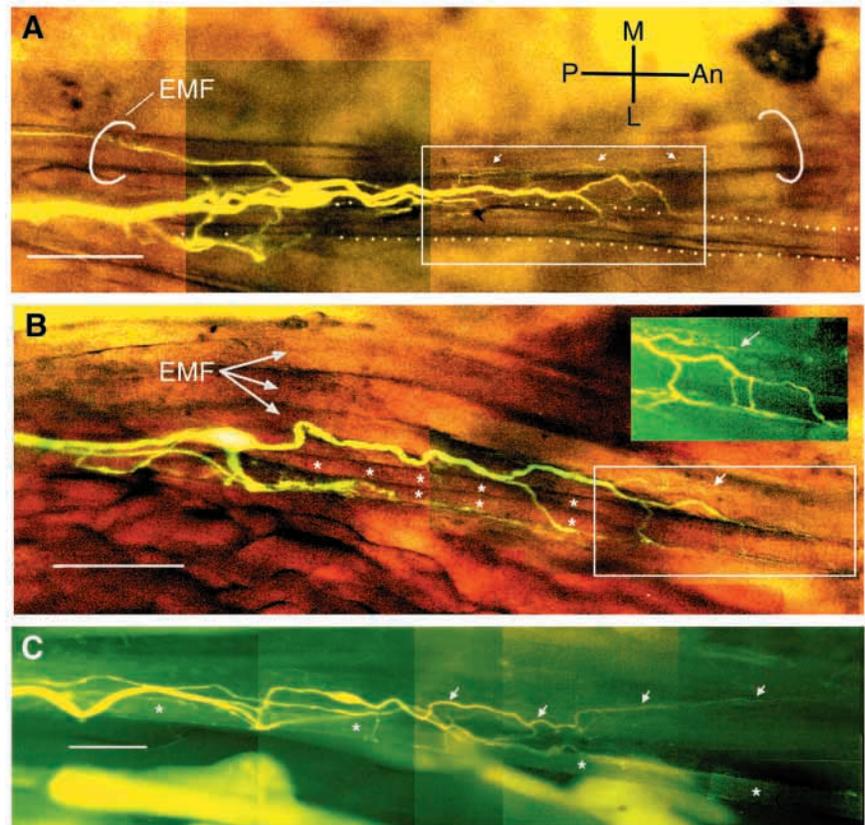


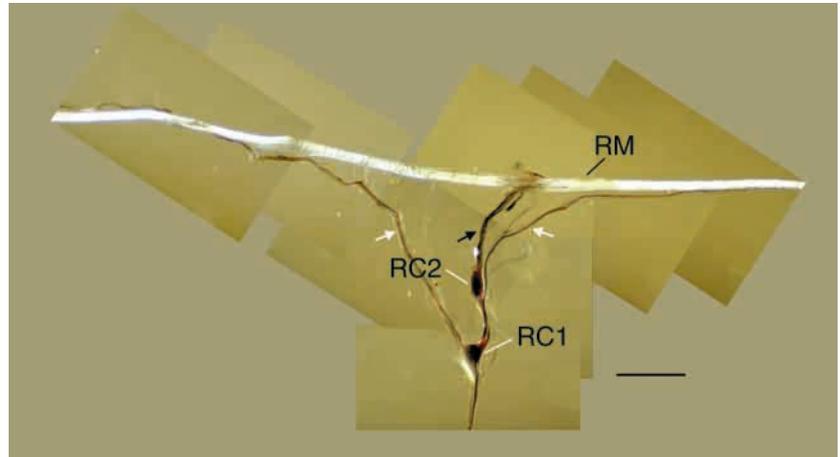
Fig. 7. Three examples of TSR-2s filled with Lucifer Yellow. (A) This example was photographed simultaneously under normal and ultraviolet light. Two dendritic branches from the fusiform receptor cell run anteriorly in contact with the receptor muscle-like strand (dotted lines), and their fine dendritic branches terminate in the neighbouring extensor muscle fibre (small arrows within the rectangle). (B) Two anterior dendrites from a fusiform receptor cell entwine with the muscle strand (asterisks), and a fine dendritic branch on the medial side terminates in the juxtaposed extensor muscle fibre (arrow in the area enclosed by a rectangle and shown in the inset). (C) Fluorescent photomicrograph. The fusiform receptor cell gives off an anteriorly directed stem dendrite, from which a small dendrite entwines with the muscle strand (asterisks) and two dendritic branches bifurcate; one runs towards the neighbouring extensor muscle (arrows) and the other entwines with the muscle strand. In this example, an axon from the posterior pole of the receptor cell can be clearly seen. An, anterior; EMF, extensor muscle fibre; L, lateral; M, medial; P, posterior. Directions in A apply to B and C. All scale bars, 400 μ m.



or incompleteness of backfilling. The common characteristics of TSR-2 were easily recognized: the main dendritic process runs forward parallel to the muscle strand and extends thin dendritic branches that entwine with the connective tissue of the muscle strand, but the thin dendritic branches do not interdigitate with the muscle fibre, unlike those of the abdominal MRO of the crayfish. Furthermore, the thin dendritic branches partly innervate the neighbouring extensor

muscle (arrows in Fig. 7A–C). The dendritic processes of the receptor cell of TSR-2 also run posteriorly along the muscle strand (Fig. 7A). Around the posterior region of the cell body, the receptor cell of TSR-2 gives off an axon, which extends through the dorsal nerve and enters N3 of the second thoracic ganglion. The axon bifurcates at the root of N3 into a descending and an ascending branch (Fig. 5B), as do the axons of abdominal stretch receptors in crayfish (Bastiani and

Fig. 8. A NiCl_2 fill of TSR-3, which consists of a single receptor muscle (RM) and triangular (RC1) and fusiform (RC2) receptor cells. White arrows indicate dendrites of RC1, which extend along the entire receptor muscle in a V-shaped manner. The black arrow shows the stout dendrite of RC2, which terminates in the central part of the receptor muscle. Since the receptor muscle is long, it is cut off at both sides. This photograph was made under a polarised light microscope. The receptor muscle shows birefringence. Scale bar, 400 μm .



Mulloney, 1988). The ascending axon of the receptor cell of TSR-2 reaches the caudal area of the tritocerebrum through the circumoesophageal connective and forms a small terminal field (Fig. 5). We could not trace the descending axon to its terminal site because of the large size of the CNS in this animal, but in 20 examples of Lucifer Yellow filling to N3 of TSR-2, Lucifer-Yellow-labelled axons could be traced to the fifth thoracic ganglion.

TSR-3 to TSR-7

TSR-3 is a thoracic stretch receptor with a real receptor muscle and consists of a pair of receptor cells and a single, long receptor muscle (Fig. 8). The receptor muscle of TSR-3 spans the third and fourth thoracic segments. The anterior insertion of the receptor muscle of TSR-3 lies on the anterior ridge of the third thoracic segment, and the posterior insertion lies on the anterior ridge of the fifth thoracic segment. Alexandrowicz (Alexandrowicz, 1967) first pointed out this organisation of a receptor muscle in *Ligia oceanica*. The cell bodies of TSR-3, unlike those of TSR-1 and TSR-2, are oriented vertically with respect to the receptor muscle. The dendrites from the two types of receptor cells contact the long receptor muscle; one is a triangular cell (RC1 in Fig. 8) and the other a fusiform cell (RC2 in Fig. 8). RC1 gives off two long, thin dendrites (white arrows in Fig. 8). Each branch extends over half the total length of the receptor muscle, and many fine branches from these branches end in the receptor muscle. The stout dendritic process that issues from RC2 terminates in the central area of the receptor muscle and intercalates into the receptor muscle, like the abdominal MRO of crayfish. The remaining TSRs (TSR-4 to TSR-7) are very similar to TSR-3 with regard to morphology, except for the length of the receptor muscle. For example, the receptor muscle of TSR-4 spans the anterior ridges of the fifth thoracic segment and the anterior ridges of the sixth thoracic segment. TSR-4 is approximately half the size of TSR-3 in the relaxed condition (Fig. 5). The last thoracic stretch receptor, TSR-7, monitors the joint between the eighth thoracic segment and the first abdominal segment, since the anterior insertion of the receptor muscle of TSR-7 attaches to the anterior ridge of the

eighth thoracic segment and its posterior insertion attaches to the anterior ridge of the first abdominal segment.

Physiology

Stretch-activated response of thoracic stretch receptors without a central connection: TSR-1 and TSR-2

Recordings were made from the peripheral cut end of N3 of the first and second thoracic ganglia. Stretch-activated TSR-1 showed a slowly adapting response with a transient phase (inset in Fig. 9A); in its response to a large stretch stimulus, we observed saturation with regard to the impulse frequency

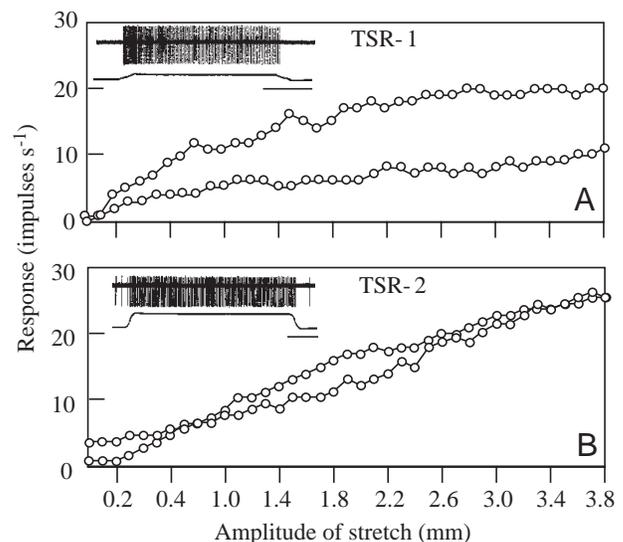


Fig. 9. Response characteristics of TSR-1 and TSR-2 to stretch stimuli. Both TSR-1 (A) and TSR-2 (B) (see insets) show slowly adapting impulse discharges in response to a stretch stimulus (time scales: 3 s in A and 5 s in B) but, as seen from the impulse frequency/stretch amplitude relationship, TSR-2 has a wider dynamic range than TSR-1. The impulse frequency/stretch relationship of TSR-2 is linear between 0.2 and 3.8 mm. The two examples in A and B were obtained from separate animals. The plots were obtained by counting impulses during the static phase within 5 s of initiation of a stretch stimulus.

(Fig. 9A). This is because the shorter dendrites of TSR-1 ramify across the extensor muscle. The response of TSR-2 to a stretch stimulus is shown in Fig. 9B (inset); spontaneous impulse discharges are usually observed in the absence of stretch stimuli. The frequency of the impulse discharge increased during stimulation, but at the beginning of a stretch-activated response there was no striking transient phase. This was also the case for one member of paired receptor cells in the more posterior TSRs. Unlike TSR-1, the discharge frequency of TSR-2 continues to increase as the stretch stimulus increases and does not reach saturation at a length of 3.8 mm. This is due to the long muscle strand of TSR-2 (Fig. 5).

TSR-3 and TSR-4

Recordings were made from the peripheral cut end of N3 in the third and fourth thoracic ganglia. Receptors TSR-3 to TSR-7 consist of paired stretch receptors (Fig. 8) and each shows two distinct types of responses to a stretch stimulus, as illustrated for TSR-3 and TSR-4 in Fig. 10 and Fig. 11. Responses to stretching were separated into two components through a window discriminator: a phasico-tonic (Fig. 10Aii,Bii) and a tonic (Fig. 10Aiii,Biii) response. Unlike the rapidly adapting response of the MRO in crayfish, the phasico-tonic response of the TSRs of *B. doederleini* continued for as long as a stretch stimulus was applied. This is clearly shown for TSR-4 in Fig. 10B, in which the two types of stretch-activated responses lasted for approximately 3 min of maintained stretch (Fig. 10Bii,iii). However, there are differences in the responses between TSR-3 and TSR-4, apparently because of the length of each receptor muscle. The receptor muscle of TSR-3 spans two body segments and is, therefore, longer than that of TSR-4 (Fig. 5). Consequently, TSR-3 responds to a large stretch stimulus without a saturation of impulse frequency (Fig. 11A,B). In contrast, the stretch-activated response of TSR-4 either reaches a maximum or decreases in frequency at a stretch length of 3.8 mm (Fig. 11C,D). However, the difference between the responses of TSR-3 and TSR-4 to a stretch stimulus might not be due solely to the difference in the length of receptor muscles; the structural properties of the dendritic attachments to the receptor muscles, the viscoelastic properties of the two receptor muscles and the membrane properties of the two receptor cells may also be involved.

Tonic responses are derived from RC1 cells, and phasico-tonic responses are derived from RC2 cells. This was ascertained by selective destruction of the cells.

Responses of the thoracic stretch receptors under flexion

To evaluate the frequency/response relationship under segmental flexion, we imposed flexion on the

animals in semi-intact preparations, as seen in the inset to Fig. 12. In this case, on the basis of the results of the behavioural experiments, flexion-induced activity was closely examined in TSR-2. As shown in Fig. 12, there was no saturation in impulse frequency in TSR-2, even beyond the maximum flexion observed in the behavioural experiment (Fig. 4).

Inhibitory intersegmental reflex

In crayfish, an inhibitory intersegmental reflex serves to control the abdominal posture (Fields, 1966, Fields et al., 1967; Page and Sokolove, 1972). We investigated whether a similar reflex occurs in the movable anterior thoracic segments in *B. doederleini*. Fig. 13 shows the inhibitory effects of TSR-3 on TSR-2 when they are simultaneously activated by stretch stimuli. This effect was obtained as follows: when the muscle

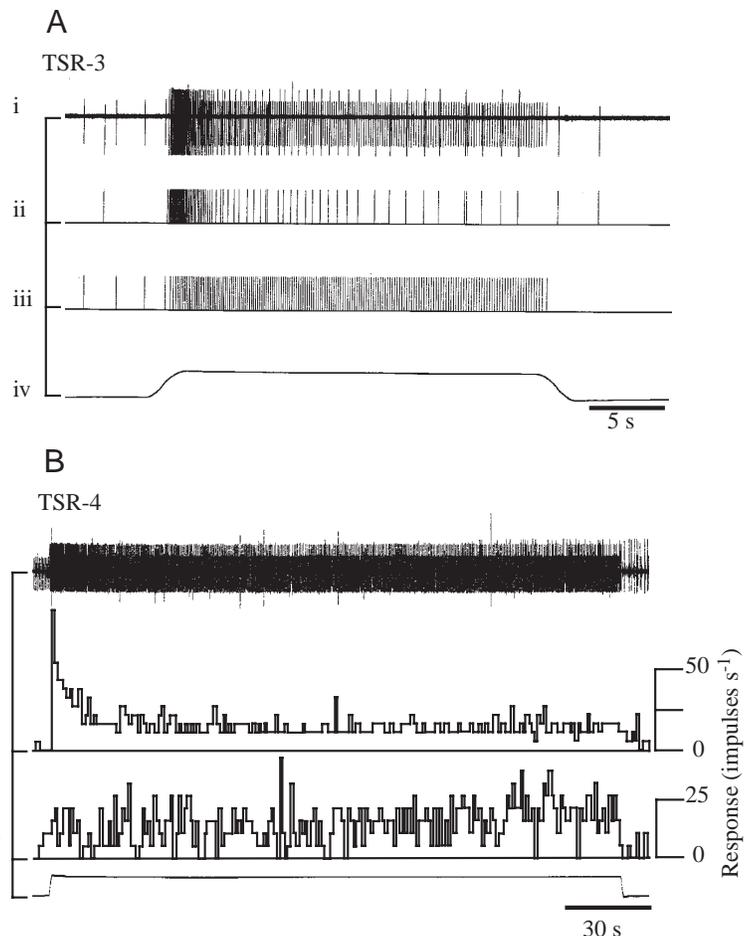


Fig. 10. Stretch-activated response of TSR-3 and TSR-4. (Ai) Two kinds of slowly adapting impulse discharges in TSR-3 (amplitude of stretch stimulus 1.2 mm). These responses were further analysed through two sets of window discriminators to show phasico-tonic (Aii) and tonic (Aiii) responses. (Bi) The stretch-activated response of TSR-4 has two kinds of slowly adapting impulse discharge: phasico-tonic (Bii) and tonic (Biii) responses (amplitude of stretch stimulus 1.5 mm). These responses were separated through the window discriminator and are illustrated as frequency histograms. Note that TSR-3 and TSR-4 responses last as long as a stretch stimulus is applied. In this case, both responses continue for approximately 3 min.

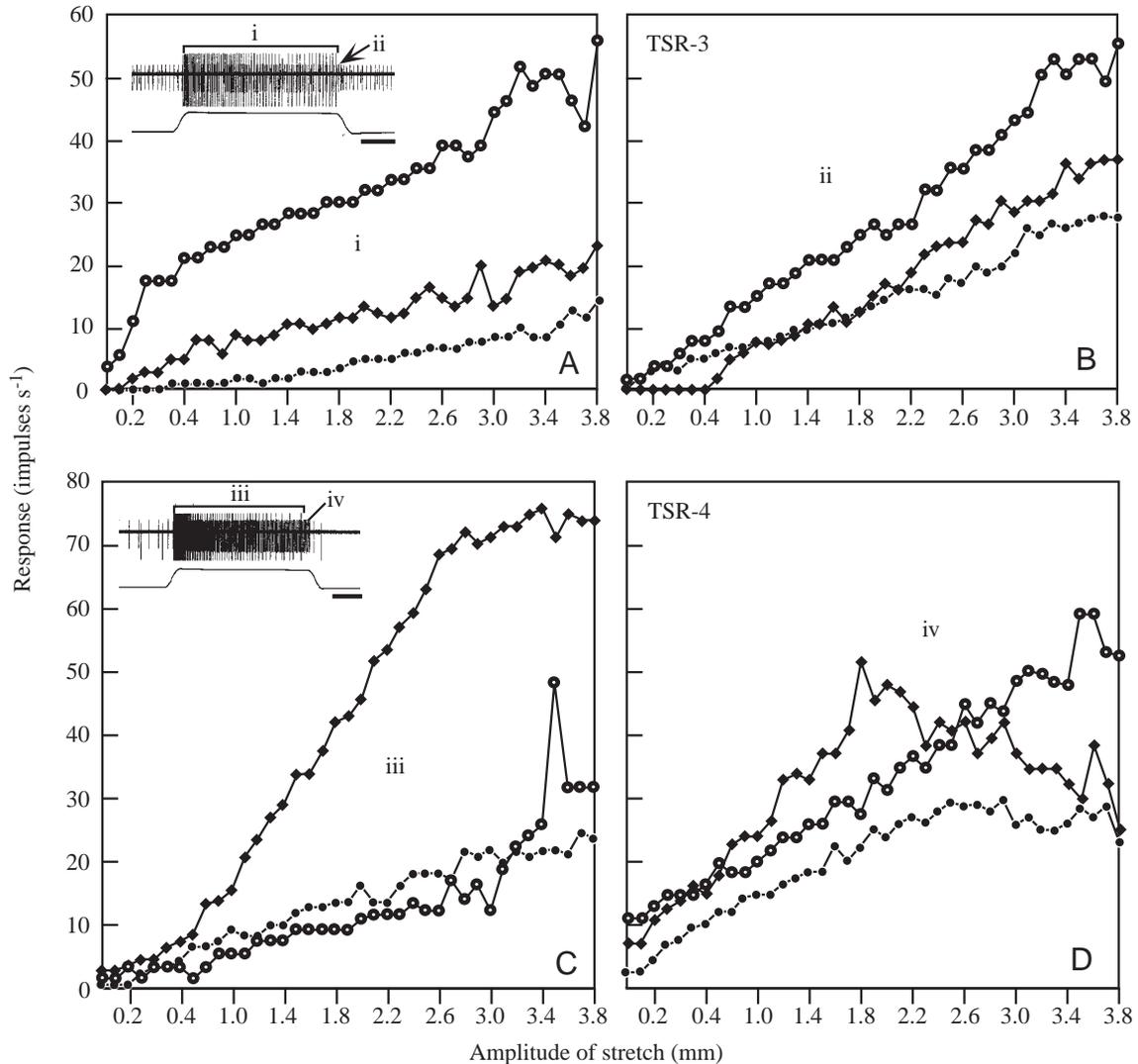


Fig. 11. Responsiveness of TSR-3 and TSR-4 to the same amplitude of stretching. (A) The inset shows stretch-activated responses of TSR-3, which consist of a phasic-tonic response (i) and a tonic response (ii). Scale bar, 10 s. The impulse frequency/stretch relationships for the phasic-tonic and tonic responses are shown separately in A and B. Both the phasic-tonic and tonic stretch receptors respond to a stretch of up to 3.8 mm without saturation. (C,D) The inset shows stretch-activated responses of TSR-4. Scale bar, 10 s. The impulse frequency/stretch relationship is presented in the same manner as for TSR-3. Both phasic-tonic (iii) and tonic (iv) responses at a stretch of 3.8 mm appear to reach a plateau impulse frequency or show a decrease in impulse frequency. The same signals indicate a pair of phasic-tonic and tonic responses from one animal.

strand on which TSR-2 lies was manually stretched with forceps (see Fig. 2B), TSR-2 discharged its stretch-activated impulses (Fig. 13A). Approximately 10 s after activation of TSR-2, the receptor muscle of TSR-3 was intermittently stretched by a vibration device (Fig. 13C). With each stretch of TSR-3, the number of impulses discharged in the preceding stretch-activated response in TSR-2 decreased (Fig. 13A,B). The second stretch stimulus with the same stretch length (1 mm) was more effective than the first, and the third stretch stimulus almost completely inhibited the response of TSR-2. With each period of inhibition, a train of small impulses occurred in N3 of the second thoracic ganglion. Although this can be seen in Fig. 13A, the portion of the recording indicated

by the double-headed arrow in A is shown on an expanded time scale in Fig. 13D where, at the right of the top trace, the spike height of the small unit is indicated by two facing arrows. This small impulse train might be reflexively produced by the cells of the CNS on which the stretch-activated impulses impinged (putative accessory neurones). A similar intersegmental inhibitory effect of TSR-4 on TSR-3 (Fig. 14) was confirmed. Both types of stretch-activated impulse discharges of TSR-3 were inhibited by repeatedly stretching TSR-4; the third and fourth stretch stimuli applied to TSR-4 completely abolished the stretch-activated phasic-tonic responses of TSR-3, while the tonic response showed only a small decrease in the number of impulses.

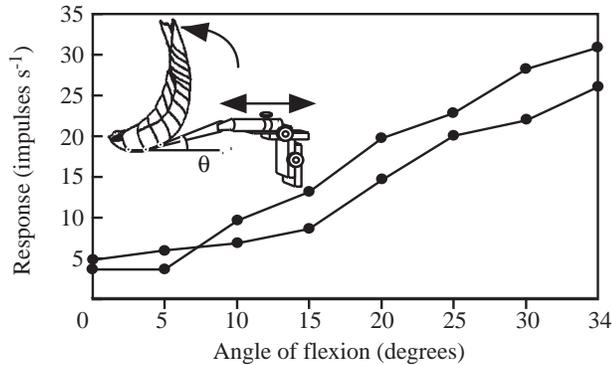


Fig. 12. Responses of TSR-2 *in situ* to flexion of the thorax. Experiments were performed on two animals. The inset shows that the anterior thoracic tergite was moved manually in the direction of the double-headed arrow. θ is the flexion angle of the second thoracic tergite, which was varied manually by means of a micromanipulator. Flexion-induced activity was recorded *en passant* from nerve 3 of the second thoracic ganglion. The anterior and posterior connectives to the second thoracic ganglion were cut.

GABA application to TSR-2

The intersegmental inhibitory reflex described above might involve γ -aminobutyric acid (GABA)-ergic neurones that may have synaptic contact with the receptor cell of TSR-2. To test the inhibitory effects of GABA on TSR-2, three concentrations were bath applied to TSR-2 in a single preparation. Inhibition of stretch-activated responses in TSR-2 was observed at GABA concentrations of 10^{-7} , 10^{-6} and 10^{-5} mol l⁻¹ (Fig. 15). In addition, we recently observed GABAergic innervation of TSR-2 using an immunofluorescent method (Cy3) (M. Iwasaki, Y. Okada and A. Niida, in preparation): a GABA-immunoreactive fibre runs along the sensory neurone of TSR-2. Its cell body and dendritic regions were covered by many GABA-immunoreactive varicosities. This finding supports the idea that GABAergic fibres innervate the receptor cell of TSR-2.

Discussion

N-cell- and MRO-type stretch receptors in isopods

Thoracic stretch receptors of isopods have features similar to those of Malacostraca. On the basis of those features, we can categorise thoracic stretch receptors into two classes: N-cell- and MRO-type. The anterior thoracic stretch receptors, TSR-1 and TSR-2 in *A. vulgare* (Niida et al., 1998), and TSR-1 (M. Iwasaki, Y. Okada and A. Niida, unpublished data) and TSR-2 in *L. exotica* (Niida et al., 1995b), appear to be homologous to the N-cells found in decapod crustaceans (Alexandrowicz, 1952; Alexandrowicz, 1956; Wiersma and Pilgrim, 1961; Macmillan and Field, 1994) and stomatopods (Pilgrim, 1964) since they have no specialised receptor muscles, a fusiform receptor cell with a long dendritic process on the extensor muscle and show a slowly adapting response to a stretch stimulus. Furthermore, the central projections of these cells, and apparently of TSR-2 in *B. doederleini*, are similar to those of decapod N-cell and MRO sensory neurones, which further supports the homology between the isopod and decapod dorsal receptors.

Our unpublished finding of intracellular recordings of stretch-activated responses and simultaneous Lucifer Yellow labelling at N3 of the second thoracic ganglion in *L. exotica* support the interpretation that the central projection pattern shown in Fig. 5B is derived from the TSR-2 axon in *B. doederleini*; the central patterns of the labelled axon of *L. exotica* overlapped the projection of the putative TSR-2 axon in Fig. 5B. Such central projections were also observed for TSR-1 and TSR-2 of *A. vulgare* by the application of Lucifer Yellow to the proximal cut end of N3 in the first and second thoracic ganglia (Niida et al., 1998). Furthermore, the application of Lucifer Yellow to the proximal cut end of N3 in the third thoracic ganglion revealed that TSR-3, an MRO-type stretch receptor, sent its axon the entire length of the CNS. The ascending branch forms a terminal field in the medial part of the tritocerebrum, while the descending branch makes a U-turn in the terminal abdominal ganglion. This projection pattern closely resembles that in the crayfish (Bastiani and Mulloney,

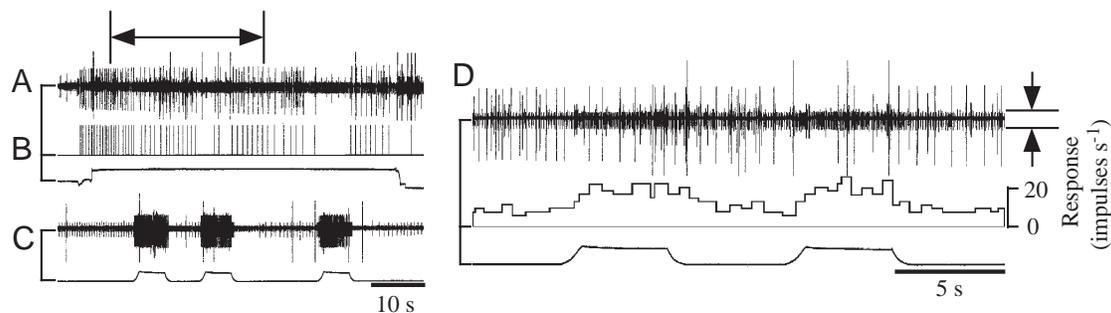


Fig. 13. Intersegmental inhibitory effect of TSR-3 on TSR-2. Stretch-induced discharges of TSR-2 are interrupted (A) by stretch-induced activity of TSR-3 (C). The upward deflection of the lower trace in C shows three consecutive stretch stimuli of 1.0 mm. These interruptions are also displayed through a window discriminator (B). The application of a stretch stimulus (1.2 mm) to TSR-2 is indicated by an upward deflection in the trace below B. (D) The region of the recording indicated by double-headed arrows in A shown on an expanded time scale. In nerve 3, small spikes (spike height indicated by facing double arrows to the right of the trace) correspond to the appearance of stretch-activated responses of TSR-3, whose responses are also presented as a frequency histogram.

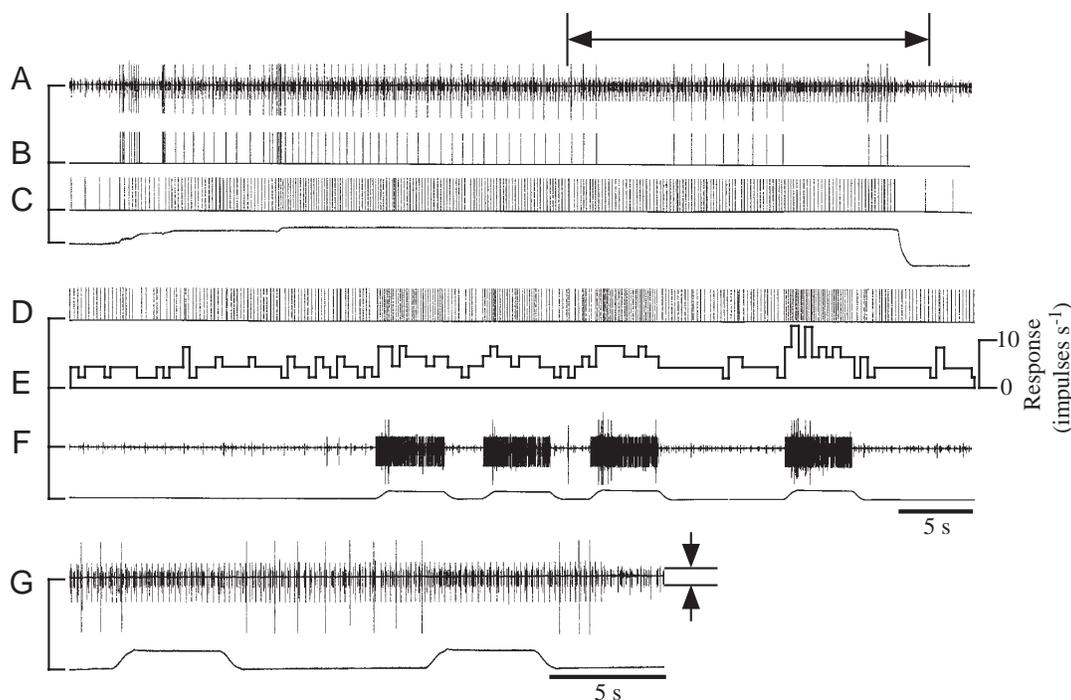


Fig. 14. Intersegmental inhibitory effect of TSR-4 on TSR-3. The receptor muscle of TSR-3 was stretched manually (upward deflection below trace C), resulting in phasico-tonic (large spikes) and tonic (middle-sized spikes) impulse discharges (A). These responses are also shown in B and C through two sets of window discriminators. Since the stretch stimulus was delivered slowly, unevenness was seen in the rising phase of a stretch stimulus. After reaching a given stretch length, the receptor muscle of TSR-4 was stretched repeatedly (F). The stretch amplitudes were given in the order 0.7, 0.7, 1.0 and 1.0 mm. The impulse discharges of TSR-3 are suppressed at a stretch length of 1 mm in TSR-4 (B), but this inhibitory effect was weaker for tonic responses (C). Impulse discharges of the putative accessory neurone through a window discriminator (D) and its frequency histogram (E). (G) The region indicated by double-headed arrows in A shown on an expanded scale. Small spikes in nerve 3 (spike height indicated by facing double arrows to the right of the trace) coincide with the appearance of stretch-activated responses of TSR-4, whose small spikes are also presented as a frequency histogram (E).

1988) and the squat lobster (Wallis et al., 1995). With regard to central projections of N-cell- and MRO-type stretch receptors, the descending component of central projections of N-cells never extends beyond the thoracic ganglia. This circumstantial evidence fully supports the presence of N-cell-type stretch receptors in isopods. The above findings were observed across taxa. Nonetheless, we feel that isopods possess cells that are homologous to N-cells in decapods.

It may be useful at this point to survey the phylogenetic relationship between Decapoda and Isopoda. Decapoda and Isopoda are not closely related, since they belong to different super-orders, Eucarida and Peracarida, respectively. However, it is thought that Peracarida are derived from a syncarid/eucarid ancestor (Dahl, 1992).

As the physiological data indicate, the dendritic branches of TSR-3 to TSR-7 undoubtedly terminate on receptor muscles. Morphologically, receptors TSR-3 to TSR-7 of *B. doederleini* closely resemble the abdominal MROs of decapods. However, there are some differences between the stretch receptors of these animals. In particular, unlike that of crayfish, the receptor muscle of TSR-3 of *B. doederleini* spans two body segments (Fig. 5) to form a single long receptor muscle. Thus, the receptor muscles in the third and fourth thoracic segments may be fused end-to-end. Alternatively, another plausible view can

be offered on the basis of the spatial organisation of the thoracic stretch receptor of *A. vulgare*. According to Fig. 2 in a previous study (Niida et al., 1998), TSR-3 of *A. vulgare* consists of two receptor muscles, each of which has one receptor cell: the short, medial receptor muscle and the long, lateral receptor muscle lie some 100 μm apart. These two muscles may have fused together in the ancestors of *B. doederleini*, resulting in a single long receptor muscle. We cannot explain the behavioural significance and functional role of this coalescence and separation of receptor muscles.

Differentiation of the anterior thoracic stretch receptor in B. doederleini

In its physiological and morphological characteristics, TSR-1 of *B. doederleini* is virtually identical to TSR-1 and TSR-2 of *A. vulgare* and *L. exotica*. However, the morphology of TSR-2 in *B. doederleini* is more specialised than those of TSR-1 and of the TSR-2 of *L. exotica* and *A. vulgare*. In *B. doederleini*, TSR-2 has a receptor-muscle-like structure that we regard as a receptor muscle strand. As seen in Fig. 7B, the muscle strand lies juxtaposed to the extensor muscle. This profile indicates that the muscle strand separates and develops from the neighbouring extensor muscle. The dendritic branches of TSR-2 are not only entwined in the muscle strand but also

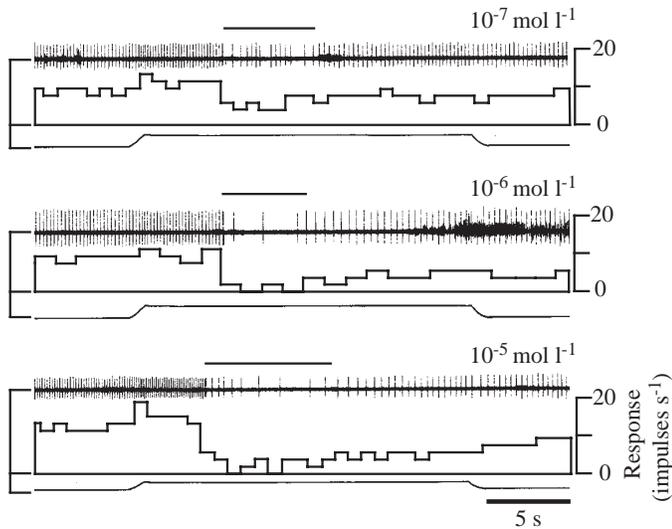


Fig. 15. Effect of bath-applied GABA on TSR-2. Inhibitory effects of GABA were observed at three different concentrations. In each experiment, GABA was applied to the same preparation after allowing it to recover from the previous dose of GABA by washing thoroughly (3 min) with normal saline. Bars indicate superfusion with GABA.

partly terminate in the neighbouring extensor muscle (Fig. 7). Such partial innervation might be an ancestral feature of anterior thoracic stretch receptors in isopods that is retained in TSR-2 of *B. doederleini*, since the long dendritic process of the receptor cell of TSR-2 in both *A. vulgare* and *L. exotica* attaches to the fascia of the extensor muscle. According to the cladogram of Isopoda (Brusca and Wilson, 1991), *A. vulgare* and *L. exotica*, as Oniscidae, are monophyletic and 'more primitive', while *B. doederleini* is a 'more derived' species. Thus, the differentiation of TSR-2 in *B. doederleini* parallels the evolutionary status of this animal. It is likely, therefore, that TSR-2 of *B. doederleini* has evolved from a TSR-1-like receptor that resembles TSR-2 found in *A. vulgare* and *L. exotica*.

Segmental mobility and structure of stretch receptors

Alexandrowicz (Alexandrowicz, 1967) suggested that whether the segmental stretch receptors of crustaceans were MRO-type or a degenerative form (N-cell) was dependent on the mobility of their segments. According to this hypothesis, both TSR-1 and TSR-2 of isopods should be MRO-type stretch receptors. This is because the thoracic segments anterior to the fourth segment are movable, as are those posterior to the fourth segment with MROs. However, TSR-1, which is situated most anteriorly, is apparently an N-cell-type, as described above. Even in the less mobile posterior thoracic segments, such as the seventh thoracic segment (Fig. 4A,B), there are MRO-type thoracic stretch receptors. Both segmental mobility and more sophisticated segmental movements might, therefore, be associated with the presence of MRO-type stretch receptors. Thus, irrespective of the presence or absence of the carapace, N-cell-type stretch receptors should commonly be found in the

anterior thorax of Crustacea. In connection with this conjecture, it would be very helpful to the understanding of the anterior thoracic stretch receptors of Crustacea if a detailed study was available of the anterior stretch receptors of comparatively primitive Malacostraca, such as *A. tasmaniaea*, which has articulated segments throughout its body (Wallis et al., 1993). We speculate that, from the primitive condition of crustaceans, there may have been two pedigrees of stretch receptors, N-cell- and MRO-types. Along these lines, we assume that TSR-2 has differentiated from the N-cell form. Recently, an alternative view was proposed in the crayfish *Cherax destructor* (Macmillan and Field, 1994): 'N-cells are derivatives of segmental repeating MROs modified to monitor postural and locomotory movements in the less mobile thorax'. This proposal substantially agrees with Alexandrowicz (Alexandrowicz, 1952) except for the interpretation of the functional roles of the receptor.

Movements of head and thoracic segments and response characteristics of anterior thoracic stretch receptors

During digging by *B. doederleini*, maximum flexions and extensions occurred between the head and thoracic segments (Fig. 4A). During rolling up, the animal showed the largest flexion around the fourth thoracic segment (Fig. 4B). The muscle strand of TSR-2 and the receptor muscle of TSR-3 are long and thus favourable for large flexion movements. These structural features reflect the responsiveness of TSR-2 and TSR-3 to stretch stimuli. For example, TSR-2 shows a wide dynamic range (Fig. 9B) and stretch-activated responses of TSR-3 followed larger stretch stimuli than TSR-4 (Fig. 11). This parallelism between response and structure is also seen for TSR-1; the small extensor muscle on which dendrites from the receptor cell of TSR-1 fan out (Fig. 6) correlates with the saturation of impulse frequency at lower ranges of stretch stimuli (Fig. 9A). Although movement of the head was not analysed quantitatively in the present study, from our observations of anterior tergal movements, it was clear that the movement of the head was consistent with that of the anterior thoracic segments. The movement of the head itself, which is independent of other thoracic segments, was less prominent.

Functional role of the inhibitory intersegmental reflex

Unlike crayfish, which have relatively immobile thoracic segments, the isopod used in this study has articulated segments throughout its entire body. Thus, our discovery of intersegmental inhibitory effects between the MRO-type receptors TSR-3 and TSR-4 (Fig. 14), which are similar to the intersegmental inhibitory reflex first demonstrated by Eckert (Eckert, 1961) in the crayfish abdomen, was not unexpected. During unrestrained flexion of the crayfish abdomen, Page and Sokolove (Page and Sokolove, 1972) clearly showed that during abdominal flexion the intersegmental inhibitory reflex mediated by accessory neurones disabled a resistance reflex. Whether a similar mechanism might be involved between TSR-3 and TSR-4 in *B. doederleini* remains to be determined. We found that the N-cell-type stretch receptor TSR-2 is also

involved in intersegmental inhibitory reflexes. The present results are inconsistent with a report that there are no inhibitory neurones in the N-cells of crayfish (Wiersma and Pilgrim, 1961). This may be due to a species-specific difference, but it should also be noted that the application of GABA (Wiersma and Pilgrim, 1961), as in the present study, to N-cells of crayfish inhibited their stretch-activated responses. A re-examination of crayfish might reveal such inhibitory neurones, since we observed inhibitory effects on N-cells in a semi-intact preparation of crayfish (Iwasaki et al., 2000).

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