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CONTROL OF ABDOMINAL EXTENSION IN THE FREELY MOVING INTACT CRAYFISH *CHERAX DESTRUCTOR*

I. ACTIVITY OF THE TONIC STRETCH RECEPTOR

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Summary

Electrical recordings were made from the sensory neurone of the tonic muscle receptor organ in the abdomen of the intact, freely behaving crayfish *Cherax destructor*. Slow extensions of the abdomen were evoked by lowering a platform from beneath the suspended crayfish, and the movements and tonic sensory neurone activity were video-recorded simultaneously. The recordings showed that the tonic sensory neurone was active when the abdomen was fully flexed prior to the extension. When the extension began, however, the sensory neurone ceased firing shortly after movement was detected, irrespective of the load applied to the abdomen. When the abdomen was physically blocked from extending fully, the sensory neurone did not fire. The tonic muscle receptor organ is considered to be the length-detecting sensor for a load-compensating servo-loop, but the results demonstrate that its activity pattern during extensions evoked by a platform-drop in *C. destructor* are not consistent with that role.

Key words: muscle receptor organ, stretch receptor, load compensation, crayfish, *Cherax destructor*.

Introduction

The abdominal muscle receptor organs (MROs) of the decapod crustaceans have been extensively studied since their original description in lobsters by Alexandrowicz (1951). However, we still have no clear picture of their functional role in the control of movement. Most studies have focused on reduced preparations with the findings then used to develop hypotheses on the role of MROs in the control of natural movements of freely behaving animals. One way to test these hypotheses is to record the activity of MRO input in fully intact, freely behaving crayfish.

Postural extension of the abdomen of crayfish is achieved by contraction of the dorsally located superficial extensor muscle and relaxation of the ventral superficial flexor muscle (Kennedy et al., 1966). The superficial extensor muscle attaches to the anterior edge of the next posterior segment to draw it forward and produce extension. The superficial extensor muscle of each hemisegment receives innervation from six superficial extensor motor neurones (SEMNs): five excitatory motor neurones and one peripheral inhibitor (Fields et al., 1967).

Closely associated with the superficial extensor muscle of each abdominal hemisegment are a tonic and a phasic MRO. Each MRO consists of a thin receptor muscle and a sensory neurone. The receptor muscle spans the abdominal joint, lying parallel to the superficial extensor muscle, and the sensory neurone embeds its dendrites into the central region of this muscle. An increase in receptor muscle tension, arising from either passive stretch or active contraction of the receptor muscle, deforms the dendrites and excites the sensory neurone (Wiersma et al., 1953). The sensory neurone of the tonic MRO is sensitive to small increases in receptor muscle tension and adapts slowly, whereas the sensory neurone of the phasic MRO responds only to a high degree of receptor muscle tension, to which it adapts rapidly. The phasic sensory neurone and phasic musculature are not active during the slow, postural movements examined in the present study (Kennedy and Takeda, 1965a,b; Kennedy et al., 1966) and will not be considered further.

Studies on the tonic MRO in isolated abdomens have demonstrated its involvement in an intrasegmental reflex in which sensory neurone discharge excites a single superficial extensor motor neurone (SEMN2) that innervates over 90 % of the superficial extensor muscle fibres in its own segment (Fields and Kennedy, 1965; Fields, 1966; Drummond and Macmillan, 1998). On the basis of this reflex, it was hypothesised that, if the joint bridged by the receptor muscle was flexed, the sensory neurone would respond and excite SEMN2 so that the superficial extensor muscle would contract until the receptor muscle unloaded, thus turning off the feedback loop. The MRO is the length-detecting element in

this resistance reflex that provides a basis for load compensation (Fields, 1966; Fields et al., 1967; Sokolove, 1973). SEMN4, a motor neurone shared by the receptor muscle and superficial extensor muscle, is capable of adjusting the tension of the receptor muscle (Fields and Kennedy, 1965).

This model, and the pattern of muscle innervation, led Fields et al. (1967) to propose two ways in which abdominal extension could be achieved. In the first, the MRO is involved in a servo-loop, and the central drive for extension would include SEMN4 to ensure that the receptor muscle contracted along with the superficial extensor muscle. In the absence of a load, the extension would proceed with both the receptor muscle and superficial extensor muscle contracting at the same rate, and the sensory neurone would remain silent. If, however, the extending abdomen encountered resistance, receptor muscle tension would develop faster than the superficial extensor muscle could unload it, and the sensory neurone would fire. The sensory neurone would increase the motor output to the superficial extensor muscle *via* SEMN2, thereby ensuring that the working muscles kept pace with tension development in the receptor muscle. The second mechanism would involve activation of motor neurones that innervate only the superficial extensor muscle. In this case, because the receptor muscle would not contract along with the superficial extensor muscle during the extension, the MRO servo-loop would be effectively bypassed, tension in the superficial extensor muscle would not be augmented and a load would cause a slower extension (Fields et al., 1967).

Studies focusing on the role of abdominal positioning interneurones in evoking behaviour support the presence of these two pathways (Fields et al., 1967; Page, 1975a). Direct electrical stimulation of these command elements activates control centres that generate coordinated motor output (for a review, see Larimer, 1988). Extension-producing interneurones, for example, act selectively upon the different efferent elements supplying the extensor muscles (Kennedy et al., 1966). In the connectives of the crayfish *Procambarus clarkii*, one type of extension-producing interneurone excites the ‘shared’ SEMN4 that innervates both the receptor muscle and the superficial extensor muscle, while other extension-producing interneurones selectively activate ‘unshared’ SEMNs that directly innervate the superficial extensor muscle but not the receptor muscle, effectively bypassing the MRO servo-loop. Recruitment of unshared SEMNs alone can only result in a centrally determined increment of superficial extensor muscle tension, whereas the use of the shared SEMN4 can provide length control (Fields et al., 1967).

Further evidence in support of the model involving the MRO servo-loop was obtained by Sokolove (1973), who recorded an increase in the firing rate of the sensory neurone when the abdomen was physically blocked from reaching its fully extended position in the intact, freely behaving crayfish. While this result implicates the involvement of SEMN4 in the extension, Sokolove (1973) was unable to confirm that this unit fired during the extension, nor could he see the sensory neurone reflexly activating SEMN2 because of the high levels of activity in other units with larger action potentials. In any case, the increased firing rate of the sensory neurone when the abdomen was blocked provides strong evidence that SEMN4 was being recruited, and it is likely that the sensory neurone–SEMN2 reflex was operational at this time.

McCarthy and Macmillan (1995) reported that the sensory neurone–SEMN2 reflex is operational in extension by analysing the movement of the abdomen before and after removing MRO input from a single segment of the crayfish *Cherax destructor*. They examined the movement of the abdomen as it extended under constant load and found a significant lag in the extension only at the joint deprived of MRO input. While this functional manipulation implicated the MRO in load compensation during extension of the abdomen, it did not provide details of the activity of the sensory neurone throughout the movement.

The present study investigates the activity of the sensory neurone in the fully intact crayfish as it generates postural extensions of the abdomen. A modified cuff electrode was used to obtain stable recordings from the dorsal nerve containing the axon of the tonic sensory neurone. The nerve recordings were acquired simultaneously with a video record of the animal’s behaviour to permit an accurate correlation between sensory neurone activity and abdominal position. The activity of the sensory neurone was assessed under a range of conditions by allowing full abdominal extension with and without constant abdominal load, and by physically blocking the extension of specific segments beyond a predetermined length.

Materials and methods

Animals and experimental preparation

Adult specimens of the Australian smooth freshwater crayfish *Cherax destructor* Clark were obtained from commercial suppliers in Victoria, Australia, and maintained in shallow indoor aquaria. The animals remained in excellent health when subjected to a normal dark:light photoperiod (12 h:12 h) and fed weekly with dry guinea-pig pellets. Sixty-eight intermoult crayfish of both sexes, with all appendages intact and with a cephalothorax length of 4.5–6.0 cm were used in the experiments.

Three or four days before an experiment, a plastic nut was cemented to the posterior dorsal thoracic carapace of the crayfish using Araldite 5 min epoxy adhesive, and the antennae were shortened to 3 cm. One day before the experiment, the animal was suspended on a plastic rod in a water-filled glass tank, and a movable platform was raised to contact the walking legs. The crayfish usually flexed its abdomen within a short period, but some animals required a tap to the telson to adopt their characteristic fully flexed posture (McCarthy and Macmillan, 1995). The platform was lowered from beneath the crayfish to evoke a slow extension of the abdomen (Larimer and Eggleston, 1971; Sokolove, 1973). Only animals with consistently smooth abdominal extensions (approximately 50 % of the animals tested) were selected for further experimentation (McCarthy and Macmillan, 1995). The

animals chosen for the loading experiments had a small wire cemented to the centre of the abdominal segment to be loaded, while those selected for the blocking experiments had small Perspex cubes cemented to the segments either side of the joint to be manipulated.

The operation and the electrode

On the day of the experiment, the animal was anaesthetised in crushed ice for 20 min and then transferred to a shallow ice-filled dish for surgery. A hole of 2 mm in diameter was drilled into the exoskeleton using a dental drill to expose the dorsal nerve. A small silver hook electrode was placed around the nerve, just distal to the nerve branch leading to the deep extensor muscle, and a small Vaseline-filled Tygon cuff (Tygon tubing pulled to a diameter of 500 μm over a naked flame and cut into cuffs of length 1 mm) was slid down the wire and over the hook to insulate it and the nerve from the surrounding blood (Fig. 1A). A mixture of Vaseline and paraffin was occasionally injected into the end of the tube using a microsyringe to increase the signal-to-noise ratio of the recording. A reference electrode was placed nearby. The electrode was maintained in this position by attaching the electrode wire to a point nearby on the cuticle. The hole was covered with the Vaseline/paraffin mixture to prevent blood loss, and the animal was placed on the platform in the water-filled recording tank for 1 h to recover from the anaesthetic. The water in the tank was earthed, aerated continuously and maintained at 17–18 °C.

The *en passant* recording from the dorsal nerve was amplified, filtered and sent to a computer for displaying and recording on Axotape 1.2.01 software. The signal was monitored simultaneously using a loudspeaker. A Sony Hi-8 video camera was positioned to film the monitor displaying the nerve recording and the crayfish simultaneously (Fig. 1B).

The experiments

The L-shaped platform was lowered by hand from beneath the suspended crayfish to evoke a slow abdominal extension. The platform moved only in the vertical plane and was lowered

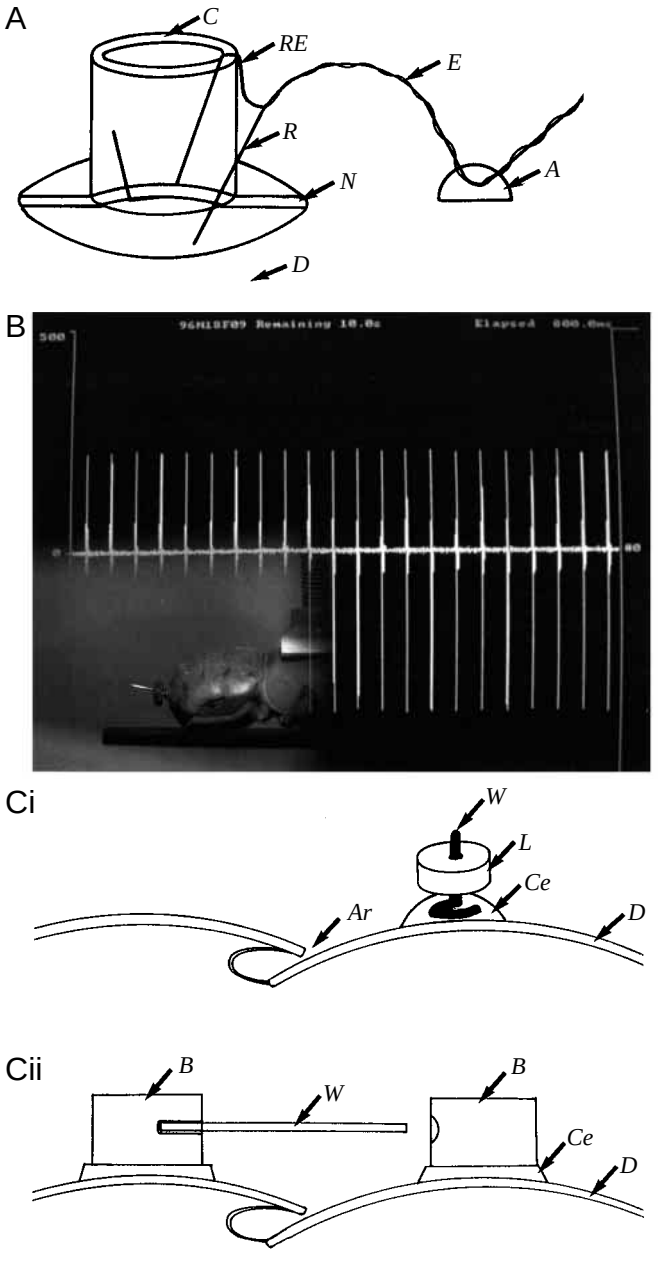

Fig. 1. (A) Diagram of the electrode used to record from the dorsal nerve in the abdomen of the crayfish *Cherax destructor*. The electrode (*E*) consists of two strands of 50 μm silver wire insulated with polyester. The wires are twisted together, and a small amount of the polyester insulation is removed from the inside of the hook of the recording electrode (*RE*) and from the tip of the reference electrode (*R*). The hook is placed under the dorsal nerve (*N*), and the Vaseline-filled Tygon cuff (*C*) slid gently down the wire and over the hook to insulate the hook/nerve junction from the surrounding blood. The nerve is maintained in this position by bending the wire of the recording electrode over the edge of the cuff, thereby giving a consistent recording of the action potentials for each unit. The attachment of the electrode wire to a point nearby on the cuticle (*A*) of the same segment ensures that the electrode moves relative to that segment and does not pull on the dorsal nerve. *D*, dorsal tergum. (B). Photograph of the image typically recorded by the video camera. The video camera is positioned to film the computer monitor displaying the nerve recording, and a mirror is positioned carefully to display the abdomen of the crayfish from a lateral perspective. In this instance, the sensory neurone for the muscle receptor organ spanning the articulation between abdominal segments 4 and 5 ($SR^{A4–A5}$) is firing tonically as the abdomen is fully flexed on the platform with a 1.3 g load attached to abdominal segment 5 (A5). Screen width, 800 ms. (C) Diagram of the lateral perspective of the abdomen of a crayfish selected for the loading/blocking experiments. Anterior is to the left. (i) Loading experiments: a small wire was cemented to the centre of the dorsal tergum of the abdominal segment to be loaded. The loads were easily secured to the segment by placing them over the wire. (ii) Blocking experiments: small Perspex blocks were cemented on either side of the joint under examination. Metal rods of differing lengths were attached to one of the cubes to block the joint from shortening to its normal extent during the extension movement. *Ar*, articulation; *W*, wire; *L*, load; *Ce*, cement; *D*, dorsal tergum; *B*, Perspex blocks.

at a constant velocity ($0.12\,m\,s^{-1}$). The extension of the abdomen was filmed together with the monitor displaying the nerve recording. The electrical recording from the nerve was also stored on the hard drive of the computer for later detailed analysis of the action potentials. After each platform-drop, the platform was raised and the animal was allowed to rest for at least 2 min before the next trial.

Two or three unhindered extensions were evoked for each experiment. In the loading experiments, a 1.3 g or 2.6 g load was secured to the segment posterior to that from which the recording was being taken (Fig. 1Ci). In the blocking experiments, a small rod, one of three different lengths (mass 0.07 – 0.12 g), was selected for insertion into one of the Perspex cubes (mass 0.1 g) to prevent the joint under examination from reaching full extension (Fig. 1Cii). Two or three extensions were generated with the load or rod treatment followed by two or three unhindered extensions. This procedure was repeated for the different treatments until at least five smooth extensions for each had been obtained.

At the completion of each experiment, the animal was killed and a *post mortem* analysis was conducted to test visually whether implanting the electrode had badly damaged the dorsal nerve. The abdomen was dissected from the ventral side to expose the region where the electrode was attached to the dorsal nerve. Visualisation of the dorsal nerve and its branches to the superficial extensor muscle was aided by staining the tissue with Methylene Blue or Janus Green B dye. In no case was visual evidence found for damage to the dorsal nerve or its branches.

Data analysis

The video recording containing both the behaving crayfish and the nerve recording was played back on the video monitor at the conclusion of the experiment, and smooth extension movements were selected for detailed analysis. A 20 ms field advance function on the Panasonic S-VHS video cassette recorder allowed an accurate correlation between the nerve recording and the animal's behaviour. The selected nerve recordings were retrieved from the hard drive of the computer, and the relevant sections, as determined from the video recording, were displayed on the computer monitor for detailed analysis. The recordings were analysed either on the computer using Axotape 1.2.01 or Axoscope 1.1 software or from plots generated using DeskPlotter 2.3 or Axoscope 1.1 software. Statistical parameters of the spike counts were analysed using a Student's paired *t*-test set at 5 % significance.

Results

Identification of the tonic muscle receptor organ

The action potentials of the units in the dorsal nerve recording were distinguishable from one another on the basis of spike amplitude and shape. The tonic sensory neurone fires large-amplitude spikes tonically when the abdomen is in the fully flexed position (see Fig. 1B). It was uniquely identifiable on the basis of spike shape and size in most experiments, and usually fired one of the largest action potentials in the recording so that its firing pattern could be easily followed even when the superficial extensor motor neurones were also active.

In the following description, each MRO is labelled with respect to the joint it spans. Thus, $MRO^{A4–A5}$ spans the articulation between abdominal segments 4 and 5, and its sensory neurone is $SR^{A4–A5}$.

Activity of $MRO^{A4–A5}$ during extension with and without load

Prior to lowering the platform, the crayfish held its unloaded abdomen in a fully flexed position. When resting in this position, the tonic $SR^{A4–A5}$ always exhibited continuous firing at approximately 20 Hz and was usually the only neurone active. Given the absence of motor activity, there could be no drive onto the receptor muscle, and the sensory neurone activity can be attributed to passive stretch of the receptor muscle (Wiersma et al., 1953). When the platform was lowered, there was an immediate burst of motor neurone activity and the abdomen began to extend. Almost as soon as the extension started, $SR^{A4–A5}$ ceased firing and did not fire for the remainder of the movement (Fig. 2A). This activity pattern was consistent in all extensions for the 15 animals examined. The sensory neurone only resumed firing when the animal returned its abdomen to a flexed position.

A similar result was obtained when a 1.3 g load was attached to A5 prior to the extension (Fig. 2B). Doubling the load to 2.6 g slowed the extension in most animals and caused noticeable difficulties for some. In spite of this, the activity pattern of $SR^{A4–A5}$ was the same (Fig. 2C).

An analysis of the activity of $SR^{A4–A5}$ prior to the extension, however, showed that, when the animal was resting with its abdomen in its fully flexed posture, $SR^{A4–A5}$ fired at a significantly higher rate when a 1.3 g load was attached to A5 (Fig. 3A). The attachment of a 2.6 g load further increased the firing rate of $SR^{A4–A5}$ (see Figs 2 and 4). However, as a result of the difficulties experienced by some animals when extending against the 2.6 g load, some animals failed to generate a sufficient number of smooth extensions for a more detailed statistical analysis to be performed.

The sensory neurone also fired more spikes after the beginning of the extension (designated as the point where the first SEMN6 unit fired; see McCarthy and Macmillan, 1999) when a 1.3 g load was attached to A5 (Fig. 3B). Although the difference was statistically significant, it should be noted that the actual number of spikes involved is small and that the sensory neurone was firing at a higher rate prior to the extension as well.

Activity of other MROs during extension with and without load

The behaviour of $SR^{A4–A5}$ was quite surprising, since it demonstrated that in *C. destructor* the sensory neurone is not signalling the load on the abdomen except prior to, and at the very start of, the extension movement. This suggests that the MRO servo-loop is not operating during these extensions. To

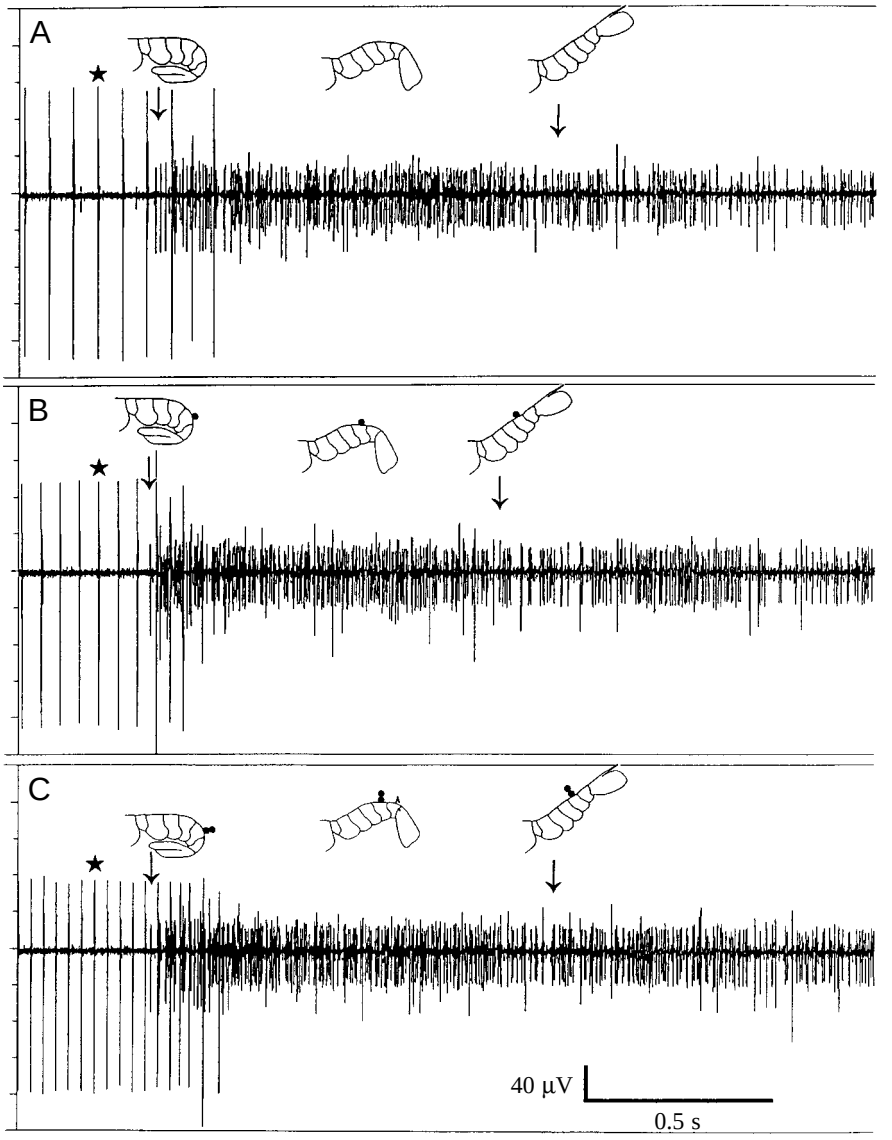

Fig. 2. Recordings from the dorsal nerve in abdominal segment 4 (A4) for one crayfish generating postural extensions of the abdomen in response to a platform-drop. The first arrow highlights the point at which the extension begins, and the second arrow marks the point at which full extension is achieved. Extensions were evoked with different loads on abdominal segment 5 (filled circles in insets): (A) no load, (B) 1.3 g load, (C) 2.6 g load. Note that the sensory neurone (SR) of the muscle receptor organ spanning the articulation between abdominal segments 4 and 5 ($SR^{A4–A5}$) (star) ceases firing soon after the extension begins and remains silent throughout the movement for all load conditions.

investigate whether the activity of $SR^{A4–A5}$ is typical of other sensory neurones, the experiment was repeated for $SR^{A2–A3}$. It was the activity pattern of this sensory neurone, recorded in *Procambarus clarkii*, that in a previous study supported the hypothesis that the MRO servo-loop operates during the abdominal extension (Sokolove, 1973).

Prior to the extension, when the abdomen was fully flexed, $SR^{A2–A3}$ fired tonically but at a much lower rate than $SR^{A4–A5}$ (approximately 3 Hz). When the extension commenced, $SR^{A2–A3}$ fired only once or twice before falling silent. This pattern of sensory neurone firing was consistent in all extensions for the 11 animals examined, whether a load was attached to A3 or not. $SR^{A3–A4}$ (three animals; load on A4) and $SR^{A5–A6}$ (three animals; load on A6) also ceased firing early in the extension movement, irrespective of the load.

Recording from nerve 2 near the ganglion

The absence of sensory neurone activity in extensions subject to constant loading suggests that under these conditions the receptor muscles of the MROs may not be receiving efferent drive in *C. destructor*. Because of the unexpected nature of this result, it was important to eliminate the possibility that the recording technique was affecting the activity of the sensory neurone. Although this type of damage was considered unlikely because the electrode was attached to the dorsal nerve just distal to the nerve branching to the deep extensor muscle and proximal to the point where the dorsal nerve begins branching to the superficial extensor muscle, and in no case was nerve damage observed *post mortem*, an experiment was designed to eliminate it as a consideration. Crayfish were anaesthetised in the usual way, and an electrode was implanted on the entire second nerve at a point close to the third abdominal ganglion. This nerve contains the axon of $SR^{A4–A5}$, but is sufficiently far from the MRO and superficial extensor muscle to exclude the possibility of any mechanical or other interference. Although the electrode recorded a large number of active units at this point, the sensory neurone was generally one of the largest units in the recording and was

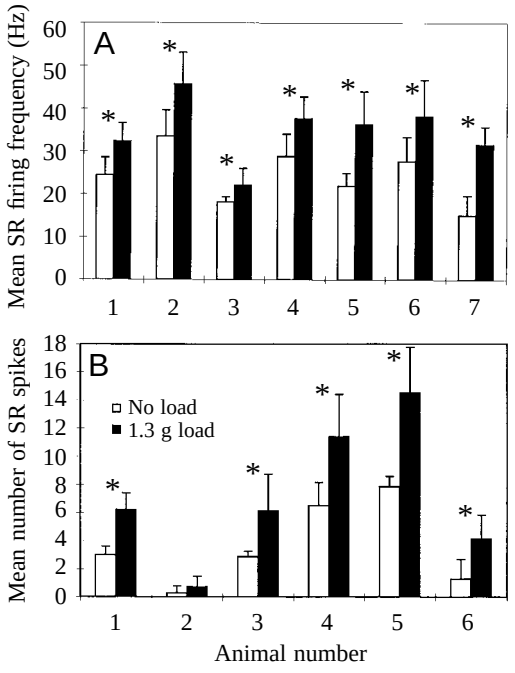

Fig. 3. Histogram comparing the firing activity of the sensory neurone (SR) of the muscle receptor organ spanning the articulation between abdominal segments 4 and 5 ($SR^{A4–A5}$) just before and just after the start of extension, with and without a load on abdominal segment 5 (A5). (A) The mean firing rate of $SR^{A4–A5}$ in the 1 s interval prior to the first action potential in superficial extensor motor neurone 6 (SEMN6) while the animal was stationary with its abdomen in its normal fully flexed position. The histograms show the results of multiple trials (means + S.D.) in seven different animals with and without a 1.3 g weight on A5. Asterisks indicate statistical significance at $P<0.05$. Animal 1, $N=7$, $P=0.0080$; animal 2, $N=6$, $P=0.0400$; animal 3, $N=9$, $P=0.0356$; animal 4, $N=11$, $P=0.0002$; animal 5, $N=16$, $P=0.0001$; animal 6, $N=19$, $P=0.0001$; animal 7, $N=7$, $P=0.0005$). (B) Mean number of spikes fired by $SR^{A4–A5}$ after the first action potential in SEMN6. Note that the sensory neurone fires only a few spikes before falling silent. The histograms show the results of multiple trials in five different animals with and without a 1.3 g weight on A5. Asterisks indicate a statistical significance at $P<0.05$. Animal 1, $N=6$, $P=0.0033$; animal 2, $N=7$, $P=0.2894$; animal 3, $N=7$, $P=0.0131$; animal 4, $N=10$, $P=0.0022$; animal 5, $N=6$, $P=0.0075$; animal 6, $N=7$, $P=0.0327$).

easily identified by its tonic activity when the abdomen was flexed. Nerve recordings in which the sensory neurone was clearly distinguishable from the other units were obtained from four animals, and in each case the sensory neurone was found to behave in a manner consistent with the earlier results: $SR^{A4–A5}$ ceased firing early in the extension movement, with or without load (Fig. 4).

Extensions in air/draining the tank water to simulate natural loading situations

Cherax destructor is able to travel over land between water bodies and so is subjected to gravity in air as part of its normal biology. To test whether the MRO responds to this greater load, two experiments were conducted in which the sensory neurone was monitored during platform-drop extensions in air. In each case, the sensory neurone fired only at the beginning of the extension in the way described previously, even though the animal struggled to extend its abdomen. In two further experiments, the water was drained from the tank after the abdomen had reached full extension. The sensory neurone was silent both before and after the water was drained.

Stopping the extension with an external rod

Given the lack of sensory neurone activity observed when the abdomen extended under constant load, where the abdomen was still able to reach its fully extended position, the technique described by Sokolove (1973) to prevent the abdomen from fully extending was applied. With this technique a stiff wire was cemented to the segment to be hindered. The platform was lowered and the abdomen extended until the wire struck a stationary rod, thereby preventing further extension. The sensory neurone was monitored in eight animals ($SR^{A2–A3}$, $N=5$; $SR^{A4–A5}$, $N=3$) as the segment posterior to that from which the recording was taken was physically stopped from reaching full extension. The sensory neurone was silent in all eight animals when the joint under examination was stopped at approximately 50 % of full extension. A disadvantage of this blocking technique in our hands was that it was difficult to block the joint under examination at a consistent length. In some trials, the stop flexed the joint beyond its normal fully flexed length, resulting in the firing rate of the sensory neurone approaching 30 Hz. A further disadvantage of the stopping technique is that it hinders the extension of segments anterior to the one that is hitting the stop. Because of the experimental ambiguities of this method, we developed a more specific blocking technique.

Blocking a specific joint with a rod attached to the animal

Sensory neurone activity was monitored in 13 animals in which a single joint was specifically blocked from fully extending during the extension movement ($SR^{A2–A3}$, $N=9$; $SR^{A4–A5}$, $N=4$). In all but one of these animals, the sensory neurone was silent when the joint was blocked at 50 % of full extension. In the remaining animal, $SR^{A4–A5}$ fired at 2 Hz in this position.

In six animals, a large rod was inserted to maintain the joint at its fully flexed length. As the joint cannot extend because of the rod, the sensory neurone would be expected to maintain its firing rate throughout the extension movement as a result of passive stretch of the receptor muscle. If the receptor muscle does receive excitatory drive under these circumstances, an increase in the firing rate of the sensory neurone would be expected. In three of the six animals examined in this way, the tonic sensory neurone maintained a steady firing rate before and during the extension movement (Fig. 5A,B). In two animals, the firing rate of $SR^{A2–A3}$ increased during the extension movement, and in one animal the firing rate of $SR^{A4–A5}$ decreased (Fig. 5A). It should be noted that these same sensory neurones were silent in all six animals when the joint was blocked at approximately 50 % of full extension.

Manually flexing the abdomen

After the abdomen had been fully extended, it was forcefully pushed into a more flexed position in seven animals. In these cases, the sensory neurone did not begin firing until the joint had been forced almost to the fully flexed position (Fig. 6). When the abdomen was manually flexed beyond the normal fully flexed length, the sensory neurone increased its firing rate dramatically, with rates approaching 100 Hz.

Other manipulations

Two additional manipulations were included to replicate the conditions applied previously to *P. clarkii* (Sokolove, 1973). In two experiments, the chelae were removed from the crayfish 1 day prior to the experiment, but the manipulation did not alter the activity of the sensory neurone during extension of the abdomen with or without load. These crayfish, however, had an increased tendency to tail-flick. In three experiments, the tank water was replaced with crayfish saline. In each of these experiments, the sensory neurone again fired in a manner consistent with the earlier experiments when the crayfish extended their abdomen with and without a load, although the extension response became progressively weaker over time.

Untethered crayfish

In two experiments, the crayfish was untethered and allowed to move freely around a small aquarium. In this situation, the animal generally walked about the tank and held its abdomen in a semi-extended position. The sensory neurone remained silent under these conditions. The crayfish also exhibited grooming behaviour and adopted the defence posture when harassed; however, the abdomen remained semi-extended during these behaviours and the sensory neurone was silent. The sensory neurone only fired when the abdomen neared full flexion. When this occurred, the rate of sensory neurone activity was typical of that seen during full flexion in tethered animals. One of the crayfish fully flexed its abdomen and then walked backwards into the wall of the tank on many occasions.

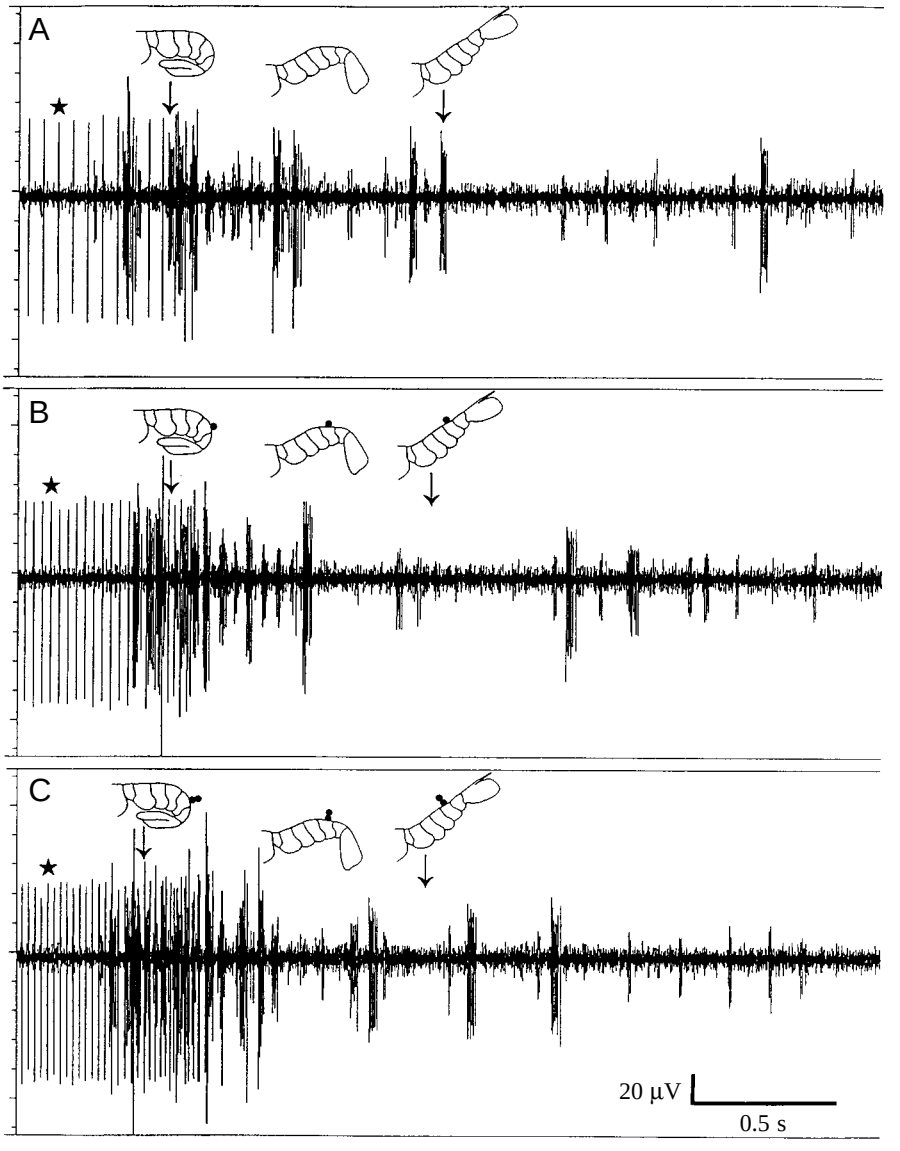

Fig. 4. Recordings from the second nerve at a point close to the third abdominal ganglion for one animal generating postural extensions of the abdomen in response to a platform-drop. Extensions were evoked with different loads (filled circles in insets) on abdominal segment 5 (A5): (A) no load, (B) 1.3 g load, (C) 2.6 g load. The first arrow highlights the point at which the extension begins, and the second arrow marks the point at which full extension is achieved. Note that the sensory neurone (SR) of the muscle receptor organ spanning the articulation between abdominal segments 4 and 5 ($SR^{A4–A5}$) (star) ceases firing soon after the extension begins and remains silent throughout the movement for all load conditions.

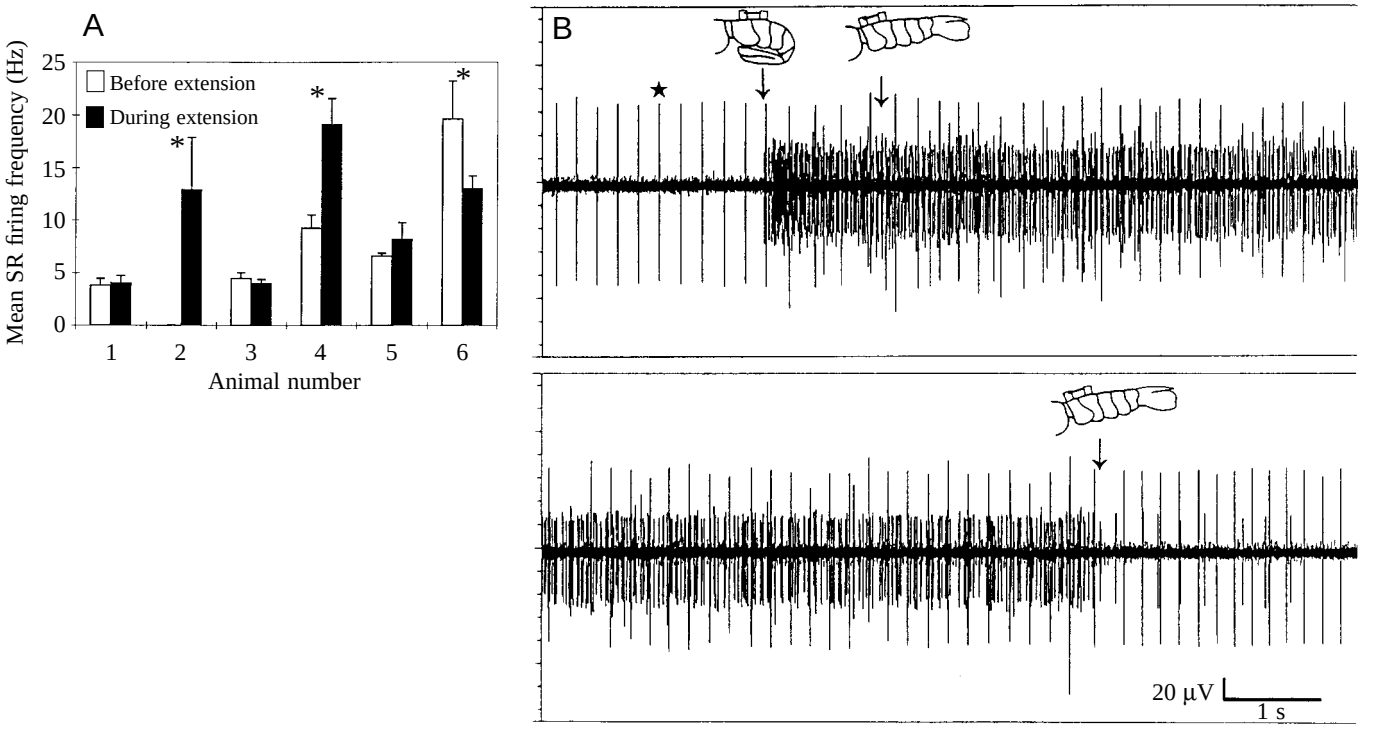

Fig 5. (A) Histogram summarising the mean firing rate of the sensory neurone (SR) before and during abdominal extension for six animals where the joint under examination was maintained at its normal fully flexed length. Note that the sensory neurone (SR) of the muscle receptor organ spanning the articulation between abdominal segments 2 and 3 ($SR^{A2–A3}$) was being monitored in animals 1–5, and $SR^{A4–A5}$ was being monitored in animal 6. The mean firing frequencies were calculated over a 1 s period just prior to lowering the platform, and the firing rate during extension was calculated over a 1 s period after the abdomen achieved 'full extension'. An asterisk indicates a significant difference at $P<0.05$: animal 1, $N=6$, $P=0.1161$; animal 2, $N=6$, $P=0.0014$; animal 3, $N=5$, $P=0.0900$; animal 4, $N=4$, $P=0.0157$; animal 5, $N=6$, $P=0.0590$; animal 6, $N=5$, $P=0.0048$). Error bars are means +1 S.D. (B) An example of an extension where $SR^{A2–A3}$ (star) fires at a consistent rate throughout the extension as the A2–A3 joint was maintained at its fully flexed length (see insets). The first arrow marks the beginning of the extension and the second marks the point where the abdomen achieves 'full extension'. The abdomen remained extended in this position until the platform was raised and the extension ceased (marked by the third arrow). The traces are continuous.

This behaviour flexed the A4–A5 joint well beyond its usual position, and $SR^{A4–A5}$ reached peak firing rates of 100 Hz.

Discussion

The tonic sensory neurone of *Cherax destructor* is not active during most of an abdominal extension induced by loss of contact of the legs with the substratum (platform-drop). Adding load to the abdomen, or placing mechanical stops that impede the movement, does not cause it to respond during extension. This is the most unexpected finding of this study because there is considerable evidence suggesting that the MRO plays a role in abdominal extension, particularly in load compensation. According to the load-compensating model, the receptor muscle of the MRO actively contracts with the superficial extensor muscle throughout extension. If resistance is encountered that prevents the joint from shortening at its centrally determined rate, tension increases in the receptor muscle, the sensory neurone fires and the afference reflexly excites SEMN2 to increase motor output to the superficial extensor muscle. The lack of sensory neurone activity during loaded and blocked extension does not support this model.

A key piece of evidence for MRO involvement in extension comes from recordings of sensory neurone activity during platform-drop extensions in *Procambarus clarkii* showing changes in its firing rate in response to a series of blocking impediments (Sokolove, 1973). In that study, as in ours, it was not technically possible to provide direct evidence of SEMN4 involvement during extension. Its presence in the motor output, however, can be indirectly demonstrated by monitoring the response of the sensory neurone. The finding that the sensory neurone of *C. destructor* was silent when the abdomen was blocked at 50 % extension contrasts sharply with the results for *P. clarkii* (Sokolove, 1973). In *P. clarkii*, $SR^{A2–A3}$ fired at approximately 40 Hz when the joint it spanned was blocked at approximately 50 % extension (see Fig. 5 in Sokolove, 1973). Furthermore, in half of all trials on *P. clarkii*, the sensory neurone fired at 60–80 Hz when the wire on the abdomen first hit the stop, irrespective of stop position. Increases of this order were never observed in the present study. The only evidence for drive onto the receptor muscle in *C. destructor* came from animals in which the joint under examination was blocked at full flexion. In some of these animals, there was a small increase in the sensory neurone firing rate during the extension.

The effect was not marked, however, and these same sensory neurones were silent when the abdomen was blocked at 50 % extension. Since the sensory neurone is highly sensitive to small length changes in the receptor muscle when at this length, the increase may have arisen from small mechanical forces from neighbouring muscles and segments (Kennedy et al., 1966; Nja and Walloe, 1975). The finding that the sensory neurone decreased its firing rate in one animal when the receptor muscle could not shorten supports this notion.

Several possibilities may account for the lack of sensory neurone activity in *C. destructor*. First, SEMN4 is not included in the extensor drive. This explanation would account for the results reported here and is supported by the presence of extension-producing interneurones which excite only SEMNs that innervate the working muscle directly, but not the receptor muscle (Fields et al., 1967; Page, 1975a). Second, the motor output includes SEMN4, but its firing rate is insufficient to cause significant tension development in the receptor muscle. Third, SEMN4 is recruited in the motor drive and causes a contraction of the receptor muscle, but the sensory neurone responds to the length of the receptor muscle alone and not to tension. A recent study by Ferber and Hustert (1996) found that the tonic receptor cell of the abdominal MRO of the locust responds only to the absolute length of the receptor muscle; when motor neurones that innervated the receptor muscle were stimulated, tension in the receptor muscle increased but the firing rate of the afferent did not change. While observations on the MROs of *C. destructor* fit this model, studies on MROs in other decapod species demonstrate that motor neurone drive onto the receptor muscle increases the firing rate of the sensory neurone (*Procambarus alleni* and *Homarus americanus*, Kuffler, 1954; *Astacus* sp., Brown, 1967; *Procambarus clarkii* and *Panulirus interruptus*, Wiersma et al., 1953; *Procambarus clarkii*, Fields et al., 1967; Sokolove, 1973). Furthermore, the afferents of the thoracic-coxal MRO in the crab *Carcinus maenas* (Cannone and Bush, 1981) and the MRO in the caterpillar *Antheræa pernyi* (Weevers, 1966) are responsive to tension changes in the receptor muscle, so this seems to be the more general case. The lack of activity in the sensory neurone of *C. destructor* is, however, consistent with other experiments on the MRO of this species. Hausknecht (1996) stimulated SEMN4 intracellularly at a high rate while monitoring sensory neurone discharge and found that the neurone had only a very weak effect on receptor muscle contraction and sensory neurone activity. Furthermore, in the present study several experiments were conducted in which the dorsal nerve was electrically stimulated extracellularly while the sensory neurone was monitored as it fired tonically, and in no case was an increase in sensory neurone activity observed (B. J. McCarthy and D. L. Macmillan, unpublished observations).

It is important when considering differences in sensory neurone responses between *C. destructor* and *P. clarkii* during platform-drop extensions to evaluate both differences in experimental methodology and interspecies differences. Replicating the blocking technique of Sokolove (1973) produced an ambiguous result in *C. destructor*. When one segment hits the stop, any or all of the joints anterior to it may

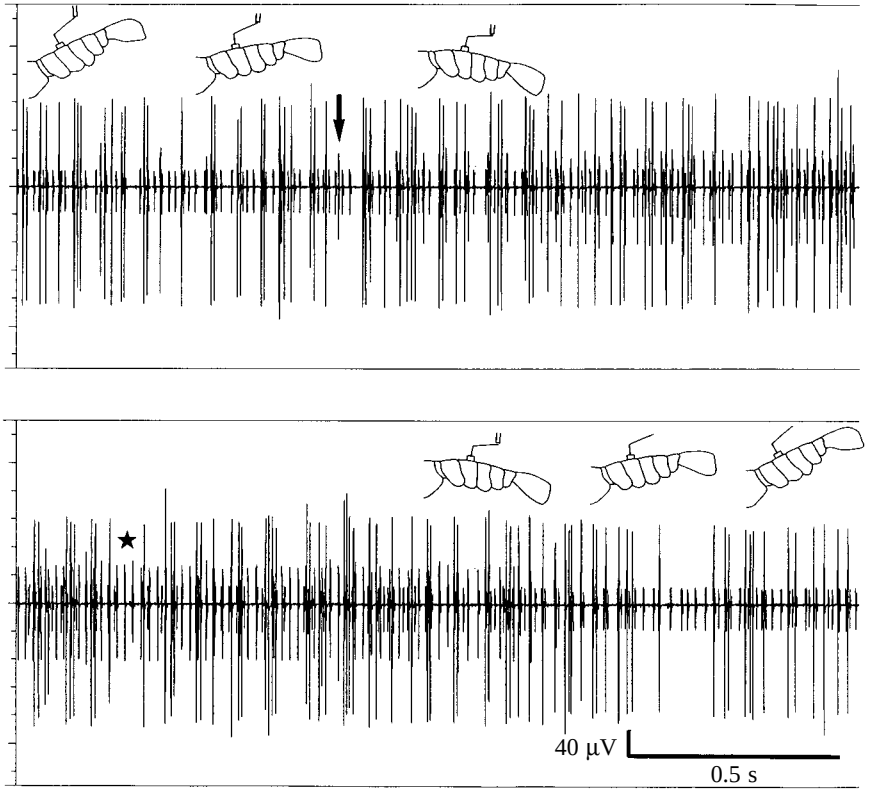

Fig. 6. Recording from the dorsal nerve in abdominal segment 2 (A2) as the fully extended abdomen is manually forced into a flexed position. The traces are continuous. In the top trace, the abdomen is fully extended (see insets) then forcefully flexed and maintained in a flexed position. Note that the sensory neurone (SR) of the muscle receptor organ spanning the articulation between abdominal segments 2 and 3 ($SR^{A2–A3}$) (first spike marked by arrow) begins to fire only when the A2–A3 joint nears its normal fully flexed position. $SR^{A2–A3}$ fires at a maximum rate of 54 Hz (region marked by a star) and ceases firing soon after the rod is removed and the abdomen allowed to re-extend.

have their extension movement altered so that changes in the behaviour of any neurones may not be attributable only to the segment being examined. It is not clear whether the experiment in *P. clarkii* could have been affected by the same problem, but possibly not, because extension forces are much larger during a *C. destructor* extension (McCarthy and Macmillan, 1995). In any case, the block failed to evoke in *C. destructor* a sensory neurone response similar to that observed for *P. clarkii*, and a key experiment would be the outcome of specifically blocking only a single joint in *P. clarkii*.

Another difference between the *P. clarkii* preparations (Sokolove, 1973) and those used in this study is that *P. clarkii* was immersed in saline with a relatively large area of cuticle removed from around the MRO. It is unlikely that performing the experiment in saline was itself the cause of any differences because this experiment was also conducted on *C. destructor* without any change in the outcome. Nevertheless, the possibility remains that exposing the MRO and superficial extensor muscle to saline removes neurotransmitters from around the MRO. The bioamines octopamine and serotonin and the neuropeptide proctolin, for example, are all present in the crustacean circulation and all significantly enhance the responsiveness of the tonic and phasic sensory neurones in *C. destructor* (Pasztor, 1989; Pasztor and Macmillan, 1990). Their absence in *P. clarkii* could perhaps account in part for a change in behaviour of the organ.

The final methodological difference between the studies is in the type of electrode used to record from the dorsal nerve. Suction electrodes were used on *P. clarkii*, while a modified cuff electrode was used on *C. destructor*. We found in the present experiments that any tension on the dorsal nerve could increase the activity of the sensory neurone, so the electrode wire was 'looped' and attached to the recorded segment to exclude the possibility of force being applied to the nerve. Suction electrodes, however, cannot be attached in this manner and are therefore generally restricted in use to the more anterior segments, which move less during extension. It may be possible that this type of electrode exerts a small force on the dorsal nerve causing the sensory neurone to increase its firing rate. The consistent results of the earlier report suggest, however, that such interference is probably not responsible for the observed result (Sokolove, 1973). Another possibility is that the differences in sensory neurone activity between *C. destructor* and *P. clarkii* during platform-drop extensions are simply interspecies differences. Although the species appear to be remarkably similar across a wide range of experimental preparations and investigations in our laboratory and others, there are differences between the two species and this possibility needs to be entertained. Two particular differences that may be germane in the present context are that the extension range of the abdomen and the velocity of extension are significantly greater in *C. destructor* (McCarthy and Macmillan, 1995; Page, 1975b).

While the tonic sensory neurone does not respond throughout the extension, it does fire while the abdomen is in the fully flexed position. Since all extensions were started from full flexion to ensure repeatability, the sensory neurone was always firing before the extension and during its initiation. It was noted that, when a load was applied by attaching weights, the rate of sensory neurone firing at full flexion increased, indicating that the MRO is detecting the load at this stage. The sensory neurone also fired more spikes during the early stage of extension (only a few action potentials, but statistically significant by comparison with the unloaded condition). It is possible that this early sensory neurone activity has some conditioning effect on the motor circuits that generate the extension. Such a mechanism would explain the finding of McCarthy and Macmillan (1995) implicating the MRO in load compensation. They showed that ablation of the MROs of one segment causes a delay in the extension of that segment relative to that of unoperated segments. The conditioning effect must have a very localised component for it to be statistically detectable at the single-segment level. McCarthy and Macmillan (1995) concluded that the MRO must be involved in a servo-loop to account for their result; however, the findings of the present study indicate that the explanation must be otherwise.

If the sensory neurone is active only when the abdomen approaches full flexion, what is its role in slow extension behaviour? Perhaps the sensory neurones act to signal the point at which full flexion is reached or to activate SEMN2 to resist large imposed flexions that may cause tissue damage. If this is the case, does the receptor muscle innervation have any role in slow extension? Perhaps the receptor muscle contracts gently together with the superficial extensor muscle so that it is not pinched between the articulations as the segments shorten. A related role has been proposed for the articular membrane and a small medial head of the superficial extensor muscle that attaches to it; the membrane muscle contracts during extension and draws forward the articular membrane to prevent it from being nipped between the sclerites (Pilgrim and Wiersma, 1963).

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