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The effect of the Crustastun™ on nerve activity in crabs and lobsters

A Scientific Report by

Professor Douglas Neil

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Introduction

The Crustastun[™] is a device designed to administer a lethal electric shock to shellfish such as crabs and lobsters before cooking, to avoid boiling a live shellfish (**www.crustastun.com**). It works by applying a 110 volt, 2-5 amp electrical charge to the shellfish. These parameters were determined by Robb (1999) and the effectiveness of the Crustastun in achieving the required stun currents was evaluated by Sparrey (2005). A previous report from this laboratory (Albalat *et al.*, 2008) evaluated the flesh quality of langoustines after being killed by the Crustastun.

The present report summarises the results obtained in a number of trials carried out to determine the effect of the Crustastun machine on activity in the nervous system of a typical crab (the shore crab *Carcinus maenas*) and a typical lobster (the Norway lobster or langoustine *Nephrops norvegicus*). On the basis of these results, conclusions have been drawn about the effects of Crustastun usage on the neuronal functioning in these and similar crustaceans.

Aims and objectives

The aims of this study were to use appropriate electrophysiological techniques to record from both the central nervous system and the peripheral nervous system of crabs and lobsters, in order to compare intact animals with those that have been subjected to 'Crustastunning'.

The specific objectives were:

1. To monitor intrinsic and evoked neuronal activity emerging from the 'brain' (supra-oesophageal ganglion) of crabs and lobsters by making extracellular recordings in the circumoesophageal connectives, the main nerves conveying information to and from the brain. This would include making recordings in the head (cephalothorax) of the lobster after isolating it from the tail (abdomen)

2. To monitor intrinsic neuronal activity in the ventral nerve cord of lobsters by making extracellular recordings from neurones in the abdominal ventral nerve cord. This would include making recordings in the tail (abdomen) of the lobster after isolating it from the head (cephalothorax).

3. To record intrinsic activity in the motor nerves leaving the abdominal nerve cord of the lobster to supply the abdominal postural muscles, by making extracellular recordings from the appropriate motor nerves (3rd abdominal roots).

4. To demonstrate evoked motor activity by measuring the muscle forces produced by the activation of the motor neurones supplying a muscle spanning a specific leg segment (the closer muscle of the dactylopodite) in crabs.

5. To determine the sensory activity in the leg nerves of crabs in response to stimulation of specific receptor types: mechanoreceptors in the cuticle (eg. cuticular hairs, campaniform sensillae) and proprioceptors spanning the leg segments internally (chordotonal organs).

These tests were designed to allow the following questions to be addressed, namely, after 'Crustastunning':

- Does any activity continue to be generated spontaneously in the central nervous systems of crabs and lobsters, and if so are its characteristics altered from normal?

- Does any activity, either spontaneous or evoked, remain in the motor and neuromuscular systems of the animals, and if so are their characteristics altered from normal?

- Does any activity remain in the sensory nerves from peripheral mechanosensory organs of the animals, and if so are its characteristics altered from normal?

Anatomy

Decapod crustaceans, the taxonomic group to which crabs and lobsters belong, have nervous systems with the characteristic arthropod plan (Brusca and Brusca, 2002). This involves a ladder-like arrangement of paired nerve cords, with a dorsal brain (supraoeophageal ganglia) separate circumoesophageal connectives and segmental ganglia in the thorax and (if present) in the abdomen, from which nerves arise to supply the segmentally-arranged muscles and sense organs. Lobsters exemplify all these features (Figure 1) whereas in crabs a distinct abdomen has been lost and the thoracic ganglia are condensed into a single thoracic mass, from which all the peripheral nerve roots emerge (Figure 2).
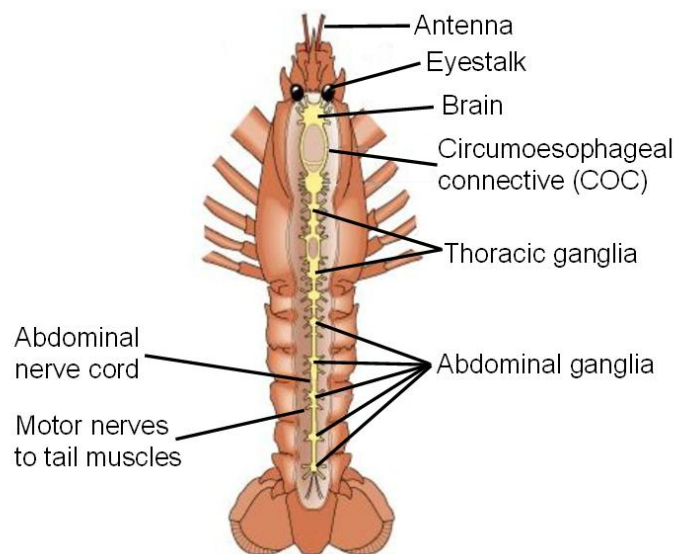

Figure 1. The arrangement of the nervous system in a clawed lobster such as the European lobster *Homarus gammarus* of the Norway lobster *Nephrops norvegicus* .
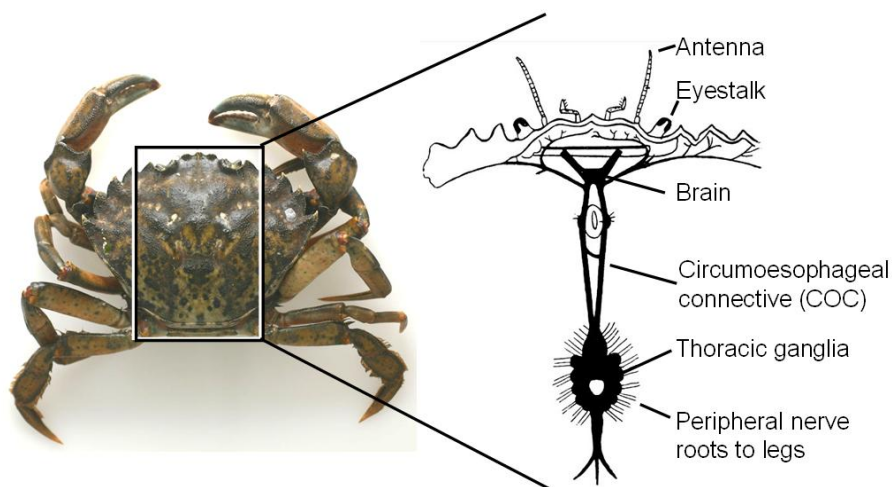
Figure 2. The arrangement of the nervous system in a crab such as the shore crab *Carcinus maenas* .

Each of the four pairs of walking legs (pereiopods) of crabs comprises a series of articulated segments, which are moved by paired muscles (Figure 3). A number of different mechanoreceptors are associated with the leg exoskeleton, including 'funnel canal organs' (a type of campaniform sensilla) which are pressure-sensitive (Libersat, 1987), and innervated cuticular sensory hairs which signal contact and water movement (Garm, 2005). In addition, a series of elastic strands span the various joints, into which are embedded sensory cells which detect joint flexion and extension (Bush, 1965). These so-called chordotonal organs thus act as proprioceptors monitoring the leg movements made by the crab (Hartman *et al.*, 1997). The chordotonal organ spanning the terminal leg segment, between the propopodite and the dactylopodite (the PD chordotonal organ) was selectively activated in this study. The branches (axons) of both the motor and the sensory nerves pass in a mixed leg nerve that travels through the centre of the leg segments.
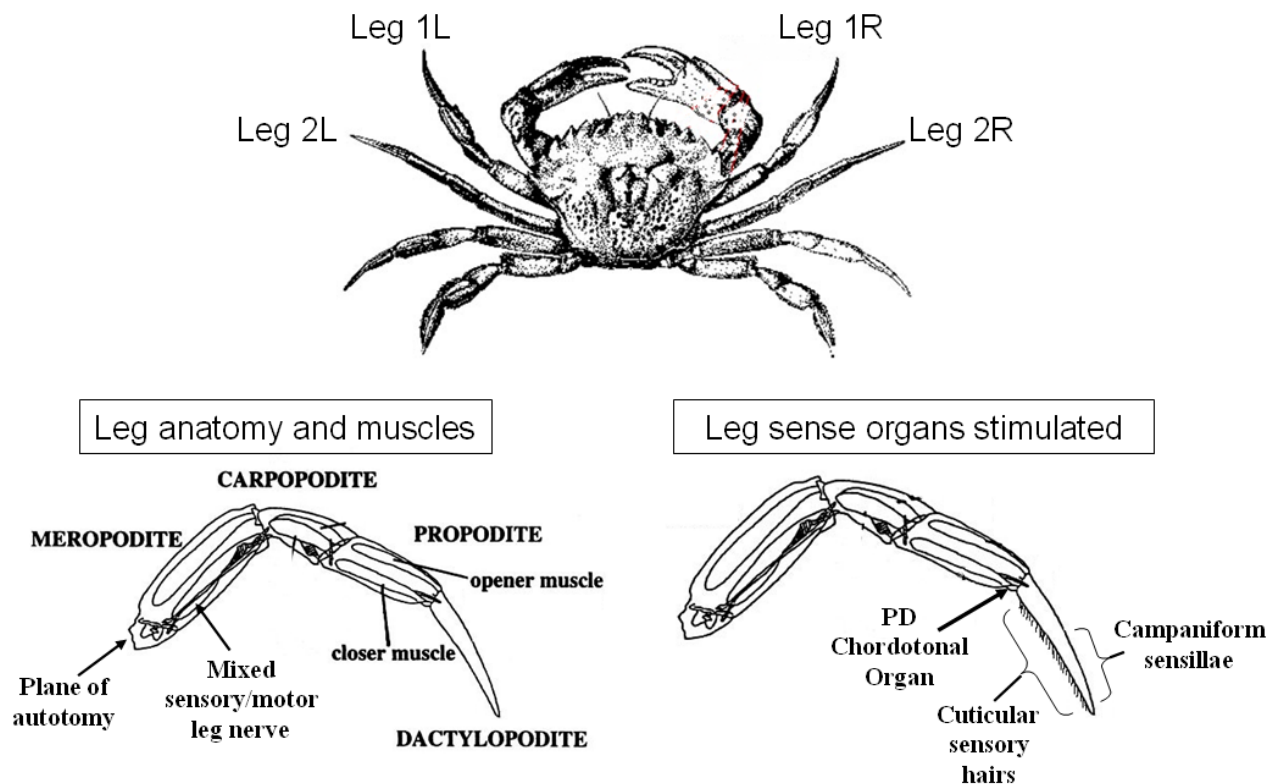
Figure 3. The anatomy of the legs of the crab *Carcinus maenas*, including the arrangement of the muscles and sense organs.

Materials and Methods

Animal supply and holding

All animals used in these tests were obtained live from commercial suppliers (UMBSM Animal Supply Service Millport and Loch Fyne Sea Farms Ltd.) and were retained within a closed seawater circulating system for at least one week before experimentation. Intact and alert animals were held on ice for 30 min immediately prior to the experimental procedures, in order to reduce their metabolic rate.

Crustastunning

The 'Crustastunning' procedure was applied without prior anaesthesia using a machine supplied by Studham Technologies Ltd., according to the manufacturer's operating instructions. The chamber was filled with a salt solution (~3g L^{-1}). Individual crabs or lobsters were stunned by a 110 volt, 2-5 amp electrical charge for 10 s immediately after removing them from the holding aquaria.

Exposing the nervous systems

In order to expose the central nervous system of the crab for recording, the carapace was removed and the preparation was submerged in a balanced salt solution corresponding in composition and osmolarity to crab haemolymph, at a temperature of 10°C. The internal organs were then removed or displaced in order to expose the circumoesophageal connectives around the base of the stomach. A similar procedure was employed for the lobster, but prior to this the cephalothorax was separated from the abdomen.

To expose the abdominal ventral nerve cord of the lobster for recording, after separating the abdomen from the cephalothorax the dorsal skeletal plates (terga) were detached, and the bulk of the underlying deep flexor musculature was removed. The preparation was then submerged in a balanced salt solution corresponding in composition and osmolarity to lobster haemolymph, at a temperature of 10°C. Selective removal of muscle blocks then revealed the motor roots emerging from the ventral nerve cord.

In order to expose the leg nerve of crabs for recording and stimulating, the leg was first detached from the body of an intact crab by applying pressure to the basipodite segment, which caused the animal to shed its leg naturally by the process of autotomy (McVean, 1976), or by amputation in a stunned crab. The joint between the meropodite and carpopidite (M-C) was then disarticulated, and the muscle tendons spanning this joint were cut with fine scissors. The leg was separated gently at this point, revealing the leg nerve still attached to the distal portion. This isolated leg preparation was submerged in balanced salt solution at a temperature of 10°C until required, and remained viable for many hours.

Electrophysiological recordings

Electrophysiological recordings were made from the exposed nerves using various extracellular techniques. For recording from the circumoesophageal connectives of crabs and lobsters, and from the ventral nerve cord of lobsters, a suction electrode method was used. A fine-tipped polythene electrode containing salt solution was applied to the surface of the nerve, and a gentle suction was applied through attached tubing and a syringe. A silver wire positioned close to the tip of the electrode acted as the indifferent (reference) electrode. Such a recording configuration is termed 'en passant', as it involves attaching the suction electrode to an intact nerve, allowing both directions of nerve transmission to be recorded. However, in some cases the circumoesophageal connective was cut and the electrode was attached to either its anterior or posterior cut end. In this way the presence of active neurones transmitting information in ascending or descending directions could be ascertained.

For recording from the crab leg nerve, the isolated leg was clamped to a Perspex plate and the nerve was passed from an adjacent bath through a wall of petroleum jelly into a second small chamber, both of which contained balanced salt solution (Figure 4, upper panel). A bipolar electrode of two silver wires was used to make contact with the solutions in the inner and outer chambers respectively.

In each case the signals from the extracellular electrodes were passed to a differential pre-amplifier (A101, Isleworth Ltd.) for amplification and filtering. The amplifier output was then passed to an Analog/Digital converter (PowerLab, AD Instruments Ltd) and was both displayed and recorded on a standard PC computer using the associated software (Chart v7, AD Instruments Ltd.)
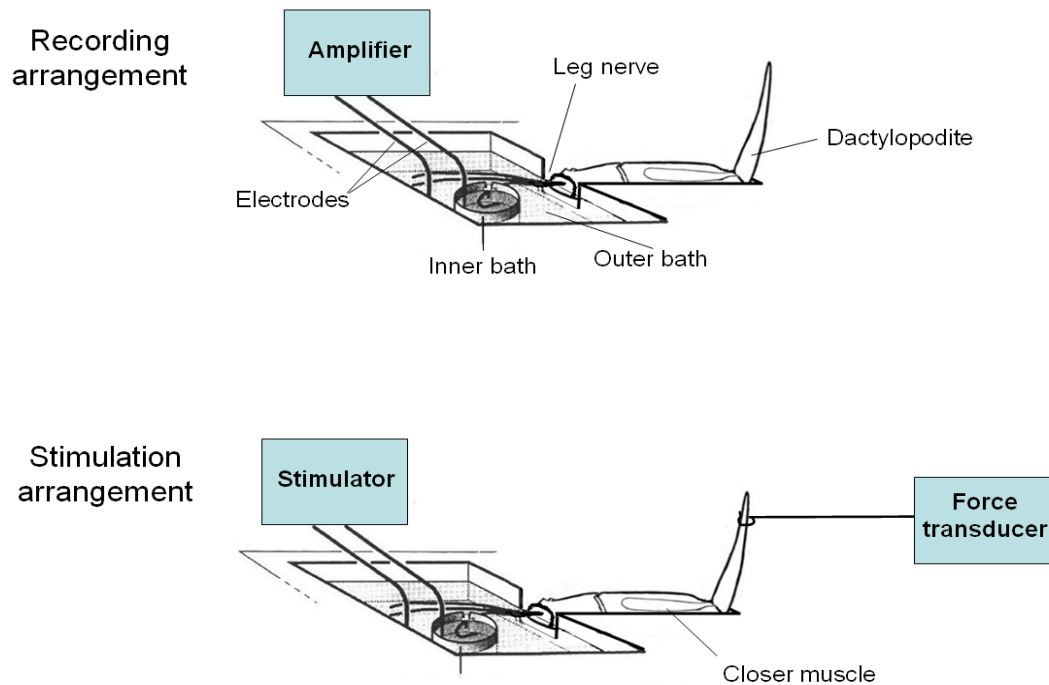
Stimulating the nerves

To stimulate the motor axons in the crab leg nerve, the bipolar electrodes were connected to an isolated stimulator within the PowerLab (Figure 4, lower panel) and patterns of stimulating pulses at various amplitudes and frequencies were applied using a software 'stimulator control panel' within the Chart v7 software. Typically, stimulus trains of 3 s duration and 10V amplitude were applied at a range of frequencies from $1 - 100$ Hz.

Recording muscle force

Although leg nerve stimulation potentially activated motor neurons supplying all of the muscles located more distally in the leg, the forces produced by the closer muscle of the Propopodite/Carpopopodite joint (P-D) were nevertheless recorded selectively. This was achieved by cutting the tendon of the antagonist muscle about that joint (the P-D opener muscle), and then attaching a thread from near the tip of the dactylopodite to the arm of a sensitive force transducer (FT-03, Grass Instruments Ltd.), mounted on a micromanipulator (Figure 4, lower panel). This selectively monitored the forces produced by the dactylopodite closer muscle. The output of the transducer was passed to a custom-

built amplifier (x1000), and then fed to an input of the Powerlab A/D converter. The forces and the stimulus parameters were then both displayed and recorded on a standard PC computer using the Chart v7 software.

Figure 4. Experimental arrangements for recording from the leg nerve of an autotomised crab leg (upper panel) and for stimulating the crab leg nerve while recording the forces produced by the dactylopodite closer muscle (lower panel).

Results

The Crustastunning procedure was applied to 6 crabs and 6 lobsters, and the same number of intact animals was used as controls. The data are presented as traces of the original electrophysiological recordings and where appropriate also as plots of the muscle forces produced in relation to stimulus parameters. Table 1 (on p. 17) summarises the results obtained.

### *Activity in the nervous system of intact crabs and lobsters*

Recordings made from one or both circumoesophageal connectives in intact crabs indicated that there was a high level of spontaneous neuronal activity passing along the axons of this nerve, even in the absence of any imposed stimulation (Figures 5 and 6). Due to the variety of sizes of the extracellularly-recorded spikes, it can also be concluded

that the signals arose from a large number of different individual nerve axons, of varying diameters.
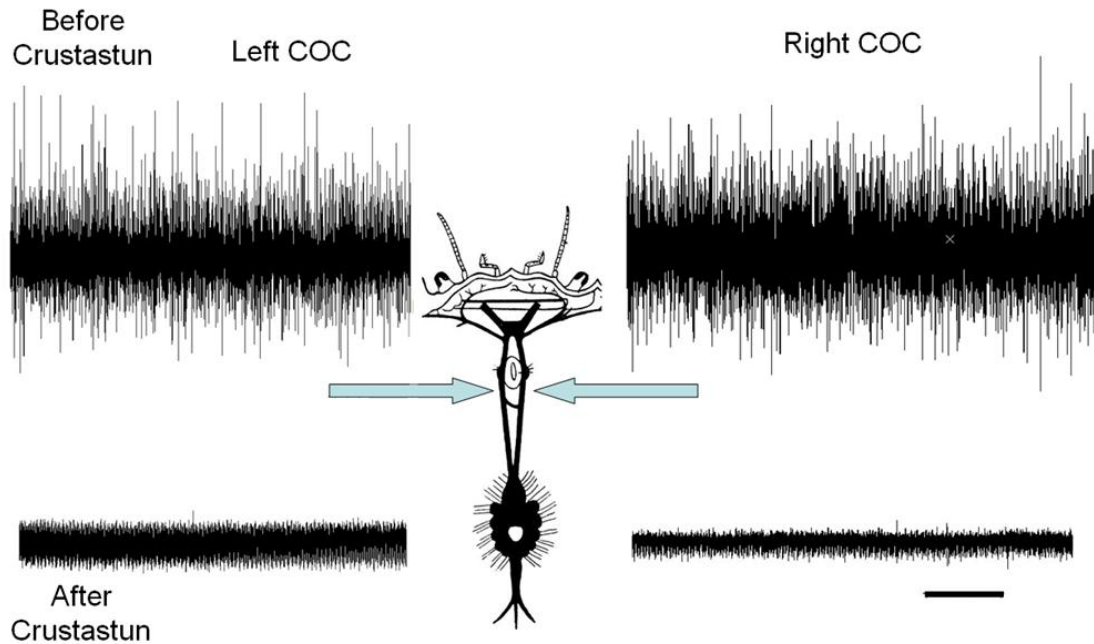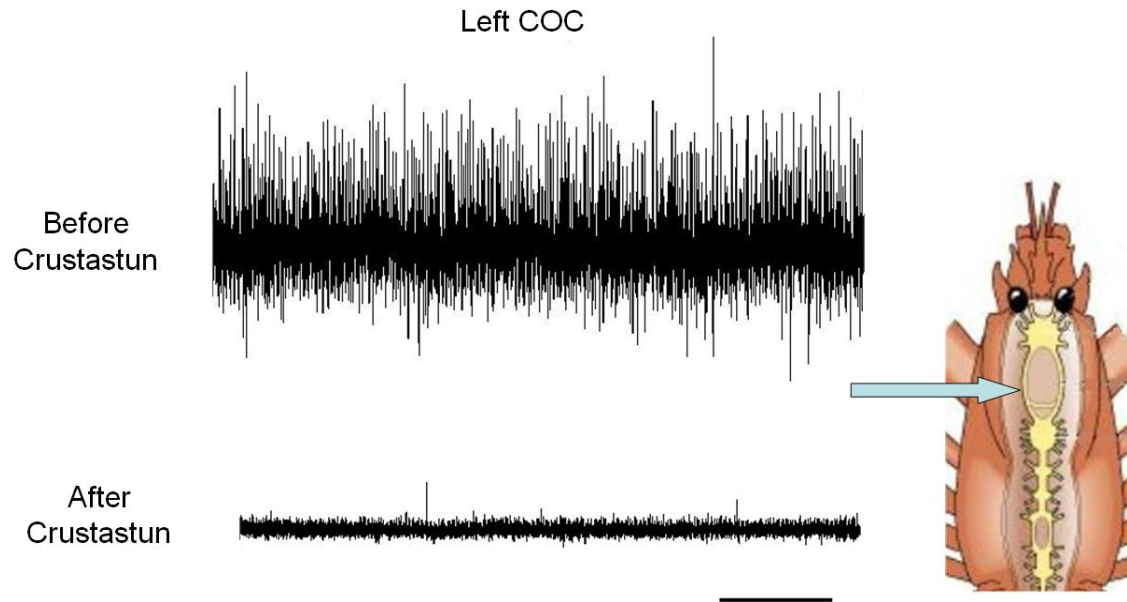

Figure 5. Spontaneous nerve activity recorded extracellularly in the left and right circumoesophageql connectives (COCs) of a shore crab, *Carcinus maenas*. Upper panel, intact animal; lower panel, animal after Crustastunning. Scale bar 1s.

When tactile stimuli were applied to the eyestalks or antennae, there were systematic changes in firing frequency in some of these axons, indicating that these were conveying descending activity from the brain. There were also high frequency bursts of activity that corresponded to the animal making struggling movements (fictive locomotion) (Figure 6), although the direction of transmission of this activity was not discernable in en passant recordings. However, recordings made from the cut ends of the circumoesophageal connectives have indicated that spontaneous activity comprises both ascending and descending nerve transmission (data not shown).
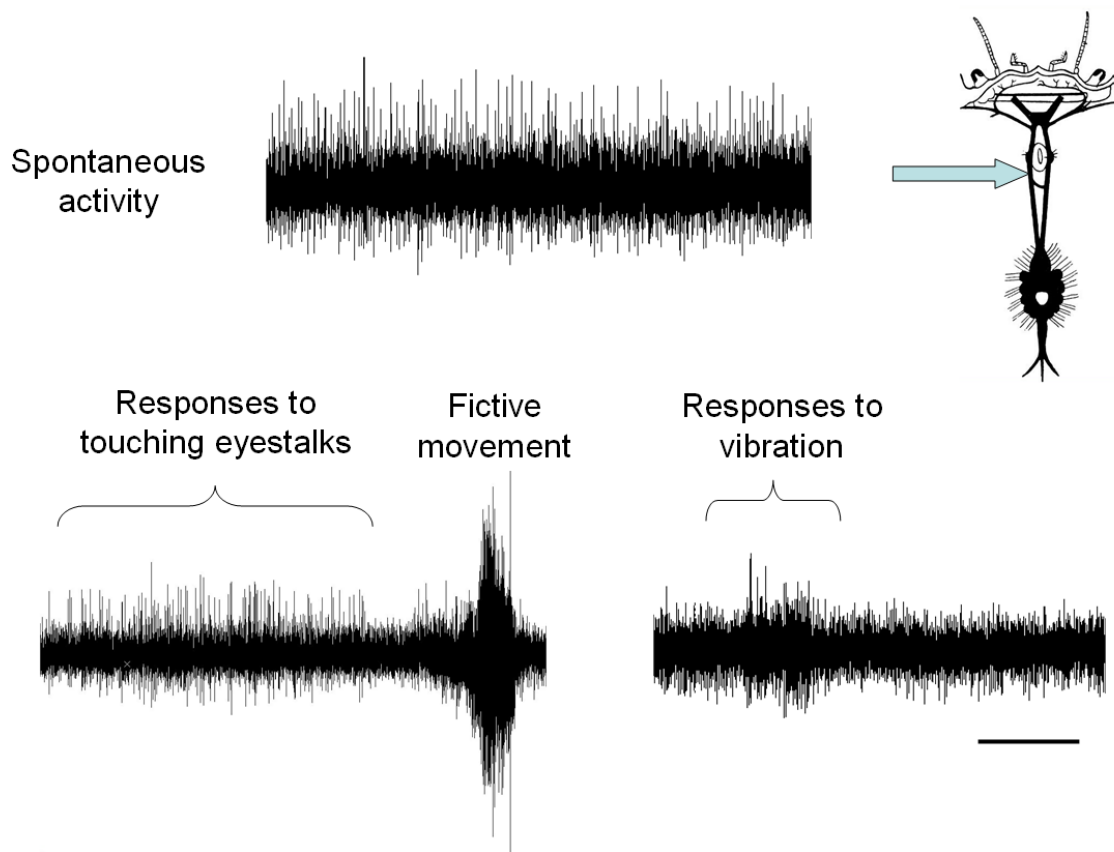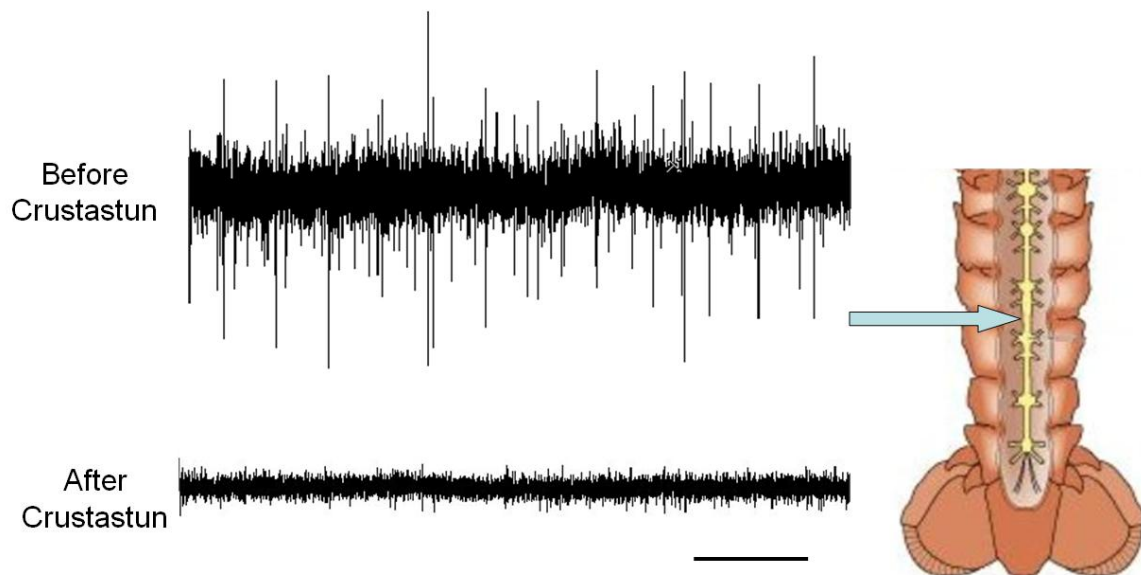
Figure 6. Nerve activity recorded extracellularly in the circumoesophageql connectives of an intact crab. Upper panel, spontaneous activity; left lower panel, responses to touching eyestalks and a burst associated with fictive locomotion; right lower panel, responses to vibration. Scale bar 2.5 s.

Recordings from the circumoesophageal connectives of intact Norway lobsters provided essentially the same results, even when the cephalothorax was detached from the abdomen, with a high level of neuronal activity passing along the axons of this nerve (Figure 7, upper panel).

Left COC

Figure 7. Spontaneous nerve activity recorded extracellularly in the left circumoesophageal connective (COC) of a Norway lobster, *Nephrops norvegicus*. Upper panel, intact animal; lower panel, animal after Crustastunning. Scale bar 1s.

Recordings from the abdominal nerve cord of the intact Norway lobsters also encountered spontaneous nerve activity in all cases, even when the abdomen was detached from the cephalothorax (Figure 8).


Figure 8. Spontaneous nerve activity recorded extracellularly in the abdominal nerve cord of a Norway lobster. Upper panel, intact animal; lower panel, animal after Crustastunning. Scale bar 1s.

Moreover, patterned activity involving a number of motor neurons (represented by different spike sizes) was detectable in all the motor roots emerging from the ventral nerve cord that were surveyed (Figure. 9). This represents evidence for the action of the peripheral nervous system in intact animals, contributing to the generation of muscle tone in the abdominal muscles.
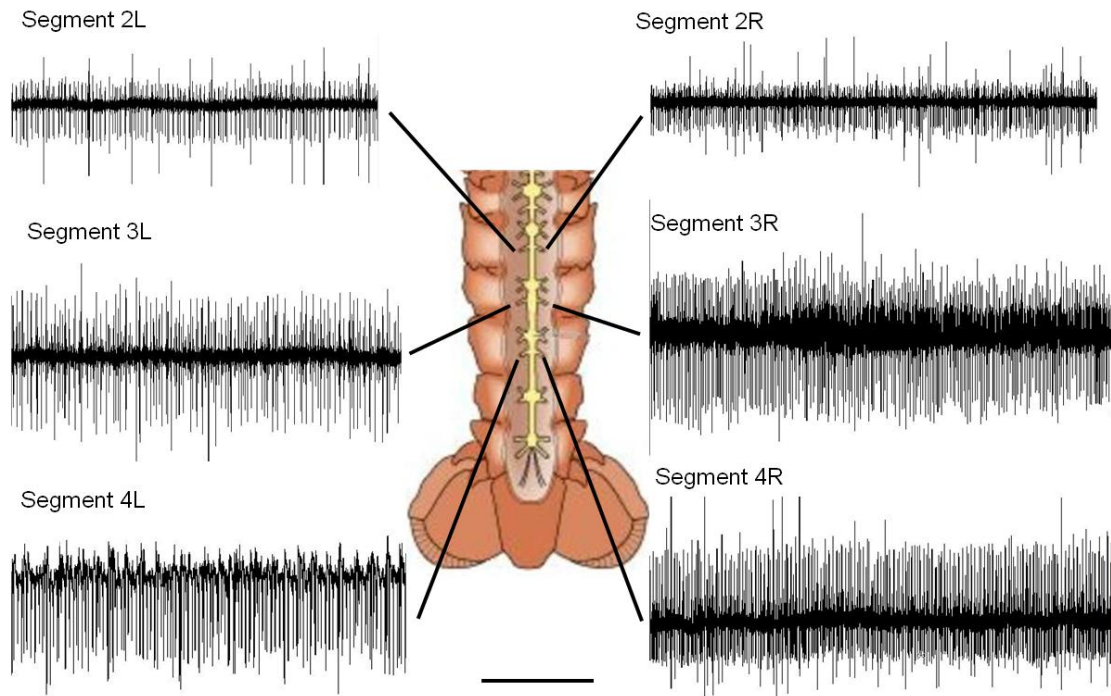

Figure 9. Spontaneous nerve activity recorded extracellularly from motor neurones in the 3rd motor roots of the abdominal nerve cord of an intact Norway lobster. The panels are from the roots indicated. Scale bar 1s.

Further evidence for activity in the peripheral nervous system in intact animals was obtained from the recordings made on the isolated legs of intact crab, following autotomy. Examples from two crabs are presented in Figures 10 and 11. The leg nerve contains a mixture of the axons of sensory and motor neurons, and the application of various stimuli to the distal part of the leg clearly elicited activity in a number of sensory neurons. These patterns of activity were typical for the various sense organs that were stimulated in each case. Thus the responses to the movement and displacement phases of flexions and extensions applied at the P-D joint had characteristic phasic and tonic elements (Figures 10 & 11, left panels). Compression (squeezing) of the cuticle of the dactylopodite elicited persistent tonic responses for the duration of the stimulus (Figures 10 & 11, centre panels). Brushing movements over the cuticle of the dactylopodite produced bursts of activity typical of the responses to displacement of cuticular sensory hairs (Figures10 & 11, right panels).
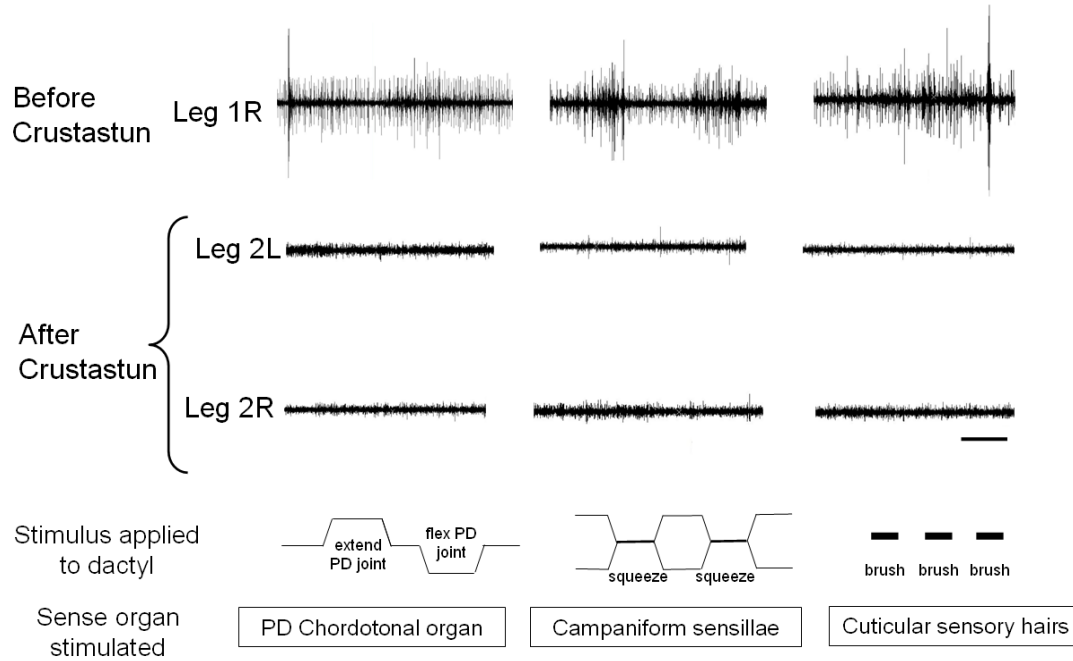
Figure 10. Responses of crab leg nerve to three forms of stimulation of the dactylopodite. Top panels, leg autotomised from intact animal; lower two panels, legs autotomised from an animal after Crustastunning. Scale bar 1 s.
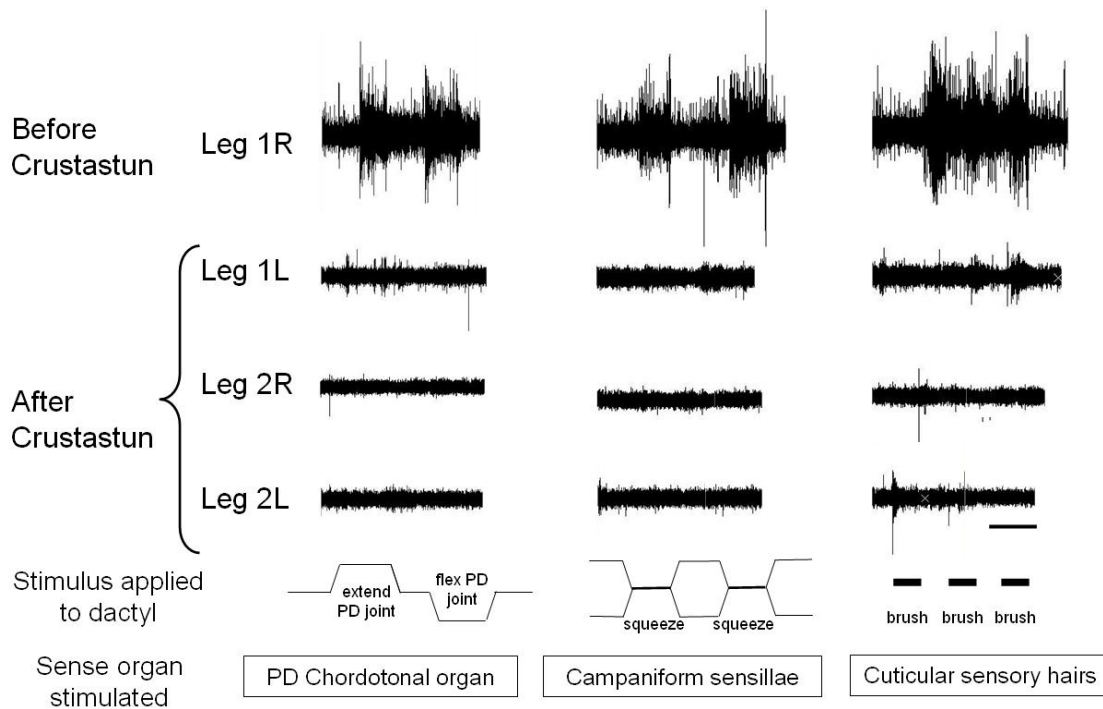

Figure 11. Responses of crab leg nerves in a different preparation to three forms of stimulation of the dactylopodite. Top panels, leg autotomised from intact animal; lower three panels, legs autotomised from an animal after Crustastunning. Scale bar 1 s.

Normal operation of the neuromuscular motor pathways in the intact crab was demonstrated by stimulating the leg nerve of an autotomised leg at a range of frequencies while monitoring the force produced by the dactylopodite closer muscle. The force varied in a non-linear frequency-dependent manner that is typical of crustacean neuromuscular systems due to their synaptic properties of summation and facilitation (Figure 12). Mean values obtained form a series of trials on 16 autotomised legs demonstrate this relationship (Figure 13).
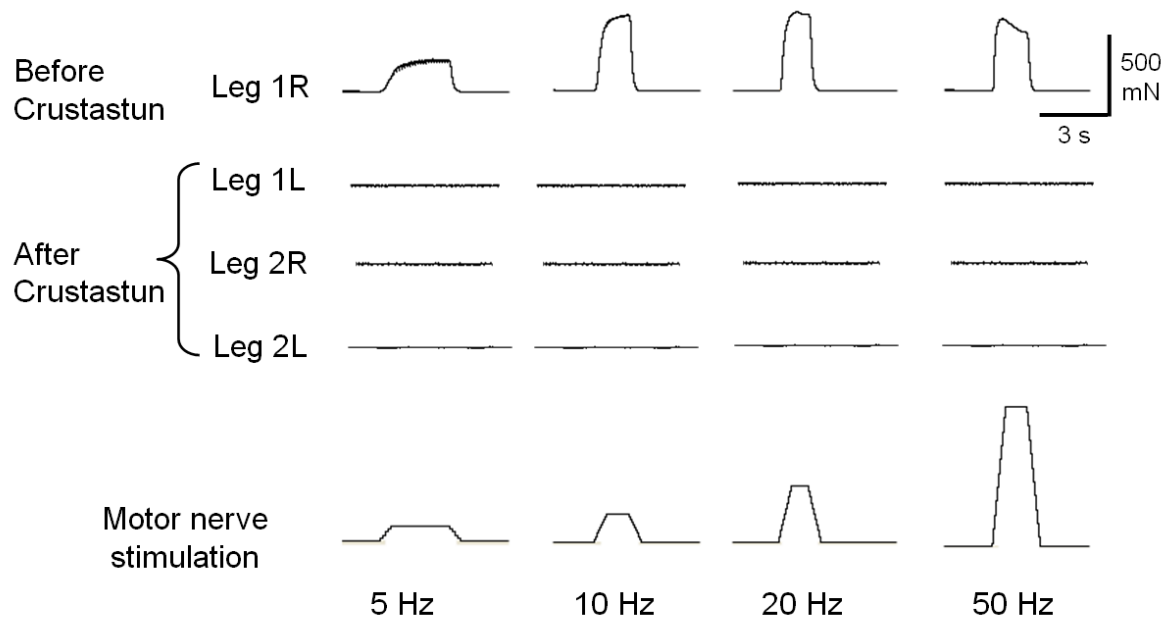
Figure 12. Forces produced by the dactylopodite closer muscle of the crab leg in response to stimulation of the leg nerve at various frequencies. Top panels, leg autotomised from intact crab; lower three panels, legs amputated from the same crab after Crustastunning. Stimulus voltage 10V.
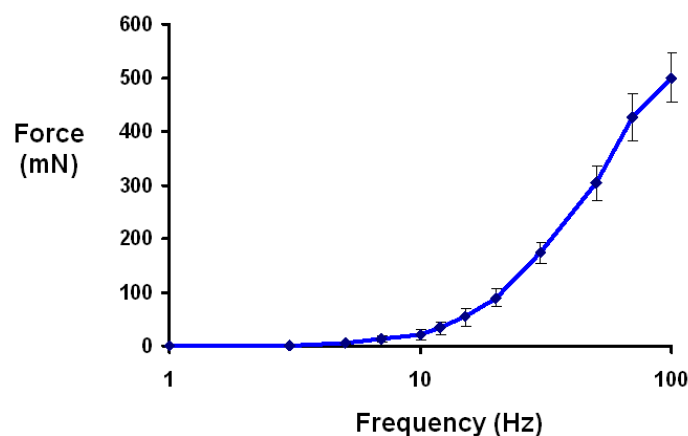
Figure 13. Forces produced by the dactylopodite closer muscle of intact crabs in relation to the frequency of stimulation (at 10V, 3 s duration). Mean ± S.E. values, N = 16 legs.

In order to test the persistence of activity in the nervous systems of intact crabs and lobsters, some preparations were re-tested at intervals of up to several hours. Activity persisted for up to the longest time tested (6 hours) in both their central nervous systems and in the nerves of automised legs (data not shown). A similar persistence was observed when a number of legs that were automised from an intact crab at the same time were held for differing periods of time before being prepared for recording (Figure 14). The sensory responses obtained at 4 h after autotomy were just as strong as those recorded immediately after autotomy.
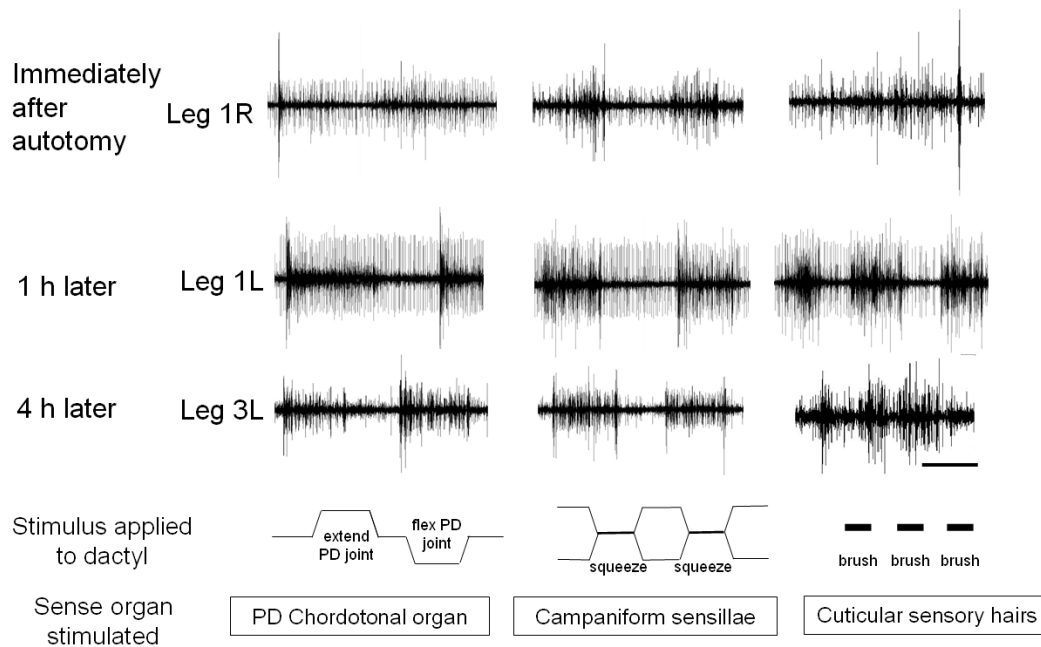

Figure 14. Responses of crab leg nerves from intact crab to three forms of stimulation of the dactylopodite at different times after autotomy. Top panels, immediately after autotomy; middle panels, 1 h after autotomy; bottom panels, 4 h after autotomy. Scale bar 1 s.

Activity in the nervous system of crabs and lobsters following Crustastunning

After Crustastunning there were no visible signs of movement in the body or appendages of the crabs and lobsters. The abdomen of the lobster was strongly flexed and lacked compliancy.

Recordings from the central nervous systems of crabs and lobsters that had been subjected to Crustastunning indicated that no neuronal activity was detectable in the circumoesophageal connectives in any of the individual animals tested of either species (Figures 5 and 7, lower panels). The abdominal nerve cords of the Crustastunned lobsters were also silent, with no indication of spontaneous neuronal activity (Figure 8, lower panel). As expected, due to this lack of central nervous system activity, there was no corresponding motor activity in the abdominal motor nerve roots of these Crustastunned lobsters (Figure 15), which contrasts with the responses obtained in an intact lobster (see Figure 9).
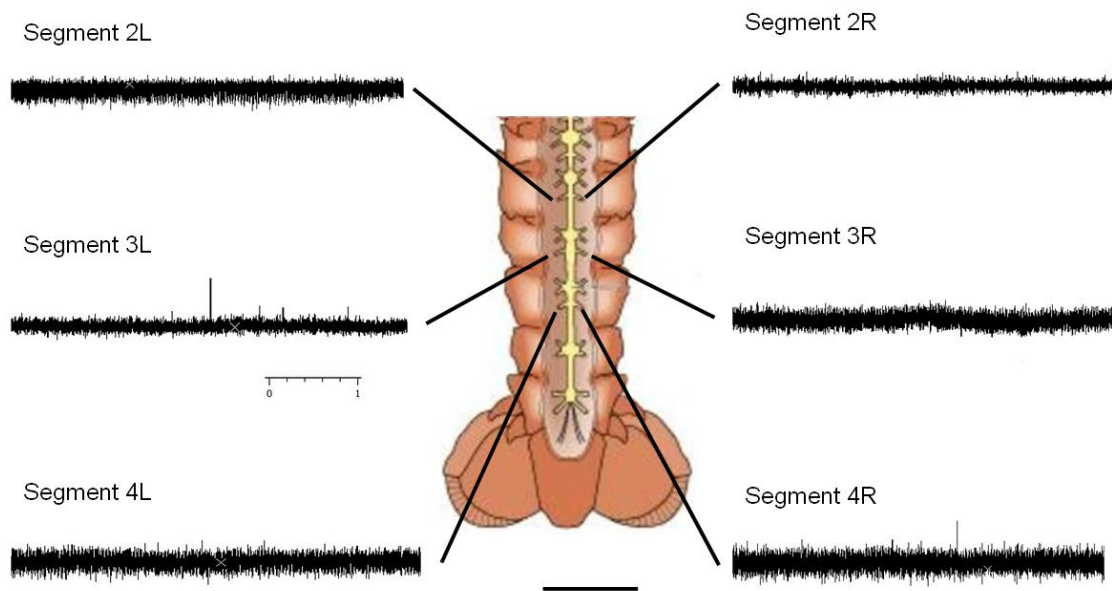
**Figure 15.** Spontaneous nerve activity recorded extracellularly from motor neurones in the 3rd motor roots of the abdominal nerve cord of a Norway lobster after Crustastunning. The panels are from the roots indicated. Scale bar 1s.

The recordings from the leg nerves of the Crustastunned crabs provided a means of testing whether the peripheral system retained any ability to convey neuronal information, even though the central nervous system might be silent. However, in virtually all the legs tested there were neither sensory responses to the three stimuli applied (Figures 10 and 11, lower panels) nor muscle force development in response to stimulating motor nerves (Figure 12 lower panels).

An exception to this was found in two individual legs from two different Crustastunned crabs. In these cases there was evidence of some recovery of neuronal responsiveness in the leg nerve over a period of minutes after Crustastunning both to sensory stimuli and to evoked motor activity resulting in muscle force development. Figures 16 and 17 show examples of this in the records obtained from one crab. In each case, as here, only one leg of the several tested from individual crabs displayed this recovery of responsiveness, while the other legs from those individuals showed no responsiveness.
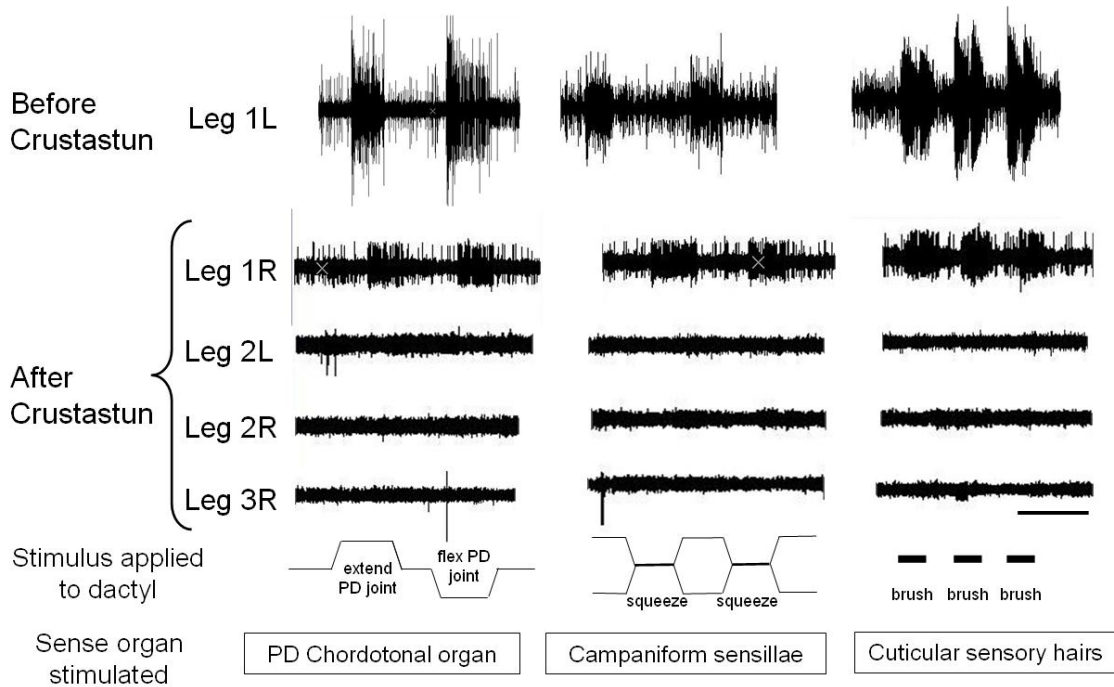
Figure 16. Responses of crab leg nerves to three forms of stimulation of the dactylopodite. Top panels, leg autotomised from intact animal; lower three panels, legs autotomised from an animal after Crustastunning. Note the continued responsiveness of the leg nerve from leg 1R, while the other legs show no responses. Scale bar 1 s.
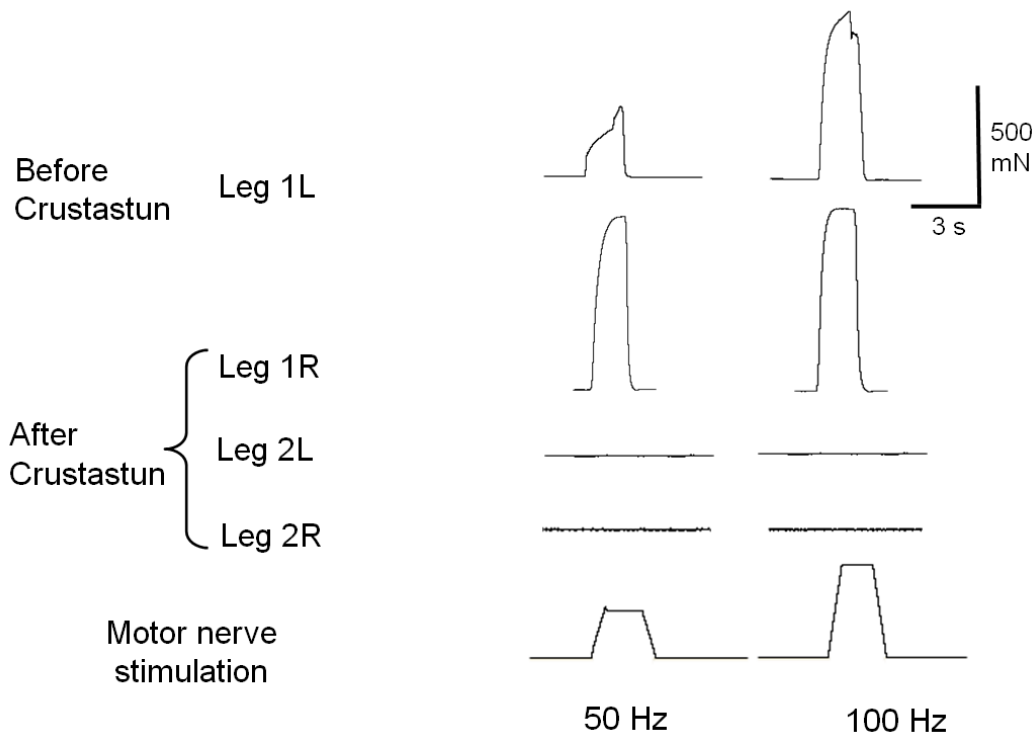
Figure 17. Forces produced by the dactylopodite closer muscle of the crab leg in response to stimulation of the leg nerve at various frequencies. Top panels, leg autotomised from intact crab; lower three panels, legs autotomised from the same crab after Crustastunning. Note the continued development of force by leg 1R, while the other legs produce no force. .Stimulus voltage 10V.

The results of the complete set of trials are summarized in Table 1. These trials were performed on a total of 6 individual animals for each treatment, and in the case of autotomised crab legs three legs per individual were tested.

Table 1. Summary of responses recorded in the nervous systems of intact and Crustastunned crabs and lobsters. Values represent numbers of animals (or autotomised legs) responding as a ratio of the number tested. n/a, not applicable.

	Intact Crab	Crustastunned Crab	Intact Lobster	Crustastunned Lobster
Spontaneous activity in Circumoesophageal Connectives	**6/6**	**0/6**	**6/6**	**0/6**
Spontaneous activity in the Ventral Nerve Cord	n/a	n/a	**6/6**	**0/6**
Spontaneous activity in the Abdominal Motor Roots	n/a	n/a	**6/6**	**0/6**
Sensory responses in leg nerve of autotomised leg	**18/18**	**2/18**	n/a	n/a
Evoked force in P-D closer muscle of autotomised leg	**18/18**	**2/18**	n/a	n/a

Discussion and Conclusions

Activity in the nervous systems

The results obtained here are consistent with the literature on the neurophysiology of crustacean nervous systems (see, for example, the articles in Wiese, 2002) in showing that the central nervous systems of crabs and lobsters display continuous nerve activity, which in turn produces outputs in the motor nerves to the body and limb muscles. A large body of evidence, including studies conducted in this laboratory (Chachri et al., 1994; Holmes et al, 2002), indicates that this activity persists even when parts of the CNS are isolated from each other by severing the nerve cord at one or more levels (Larimer and Moore, 2003). Even isolated single ganglia of the abdominal nerve cord can produce patterned outputs (e.g. Chachri and Neil, 1993), and there is an extensive literature on the most-studied ganglion that can continue to operate in isolation, the stomatogastric ganglion (reviewed by Marder and Bucher, 2007).

It is therefore not surprising to have found in the present study that, as a result of dissection or of detaching the cephalothorax from the abdomen, nerve activity continues to be recorded in the isolated anterior or posterior portions of the body, even though the nerve cord is transected at one or more levels. Also, as expected, this activity includes both descending signals from the brain and ascending signals from more posterior parts of the nervous system.

Although not attempted in these trials, it is without doubt that any procedure that attempted to make a sagittal section through a crab or lobster, in an attempt to destroy the entire nervous system, would inevitably leave small sections untouched and sufficiently intact to be able to continue generating patterned nerve activity, and to respond to sensory stimulation with reflex outputs localized to the muscles in the segments still innervated.

A characteristic feature that is common in these isolated parts of the nervous system is the long-lived nature of continued activity and signal conduction. It is widely reported that, provided the structures are bathed in an appropriate solution, activity can continue for many hours, and indeed this was observed in the present study both with the central nervous preparations and with the isolated crab legs after autotomy. Such robustness makes it easier to interpret any loss of activity following an intervention as due to the intervention itself, rather than to any underlying decline in nervous system responsiveness.

The effects of Crustatunning

The findings obtained on the effect of Crustastunning on nerve activity in crabs and lobsters are relatively conclusive. As far as can be determined from the extracellular recording method used, the various forms of spontaneous activity within the central nervous system are completely arrested. Consistent with this, there are no outputs produced in the motor nerves supplying the abdominal muscles of lobsters, which are known to be synaptically driven from neurones in the CNS.

The recordings made on isolated crab legs allow some further conclusions to be drawn, namely that Crustastunning also has an effect on the functioning of the peripheral parts of the nervous system. There is both a loss of responsiveness to all three types of sensory stimulation, and also a failure in neuromuscular activation. The first of these effects renders the animals insensitive to external stimuli, while the second renders them paralysed and incapable of making movements. Thus it has been found that as a result of Crustastunning the nervous system is incapacitated simultaneously at two levels, i.e. both centrally and peripherally, which completely prevents all normal neuronal functioning.

In terms of identifying the reasons for recording no sensory signals or inducing no motor activity in the peripheral nervous system, the recording method used does not allow definitive conclusions to be made. It is indeed possible that the conduction processes in the axons of both the sensory and motor neurones have been disrupted by the electrical currents generated by the Crustastun. However, it cannot be excluded that the Crustastunning has affected only the sensory transduction processes in the receptor endings of the sense organs, rather than the nerve transmission mechanism in the sensory nerves. Similarly, Crustastunning may have destroyed synaptic transmission at the neuromuscular junctions, and/or excitation-contraction coupling processes within the muscle fibres, rather than the nerve transmission mechanism in the motor nerves. It is of course possible that all of these processes have been affected simultaneously. To distinguish between these possibilities would require an examination of each of the contributing processes by using other, more appropriate, electrophysiological methods in a targeted approach.

The fact that neuronal integrity in the crab leg nerve persisted after Crustastunning in two instances requires an explanation. It involved both continued responsiveness to sensory stimulation and continued ability to produce motor activation of muscle contraction in response to artificial stimulation. In each case it involved only one of the legs, while the others from the same crab showed complete unresponsiveness. It is possible that in these cases one appendage was positioned within the Crustastun machine in such a way that the electrical current did not pass through it as completely as it did through the body of the animal or through the other legs. It is therefore suggested that the pathways of current flow through the animals when subjected to Crustastunning are examined in detail, and recommendations made for positioning the animal and its limbs to eliminate the possibility of such a failure to inactivate the limb nerves.

However, in terms of the consequences for neuronal action, it should be noted that the central nervous systems of animals involved in these two cases were silenced by the Crustastunning, and so despite the fact that sensory information may have reached the thoracic ganglion, this would have produced no neuronal reaction in the CNS. Likewise, although the motor pathways to the muscles were still competent, no motor commands would have been generated by the CNS to initiate movement.

Scope of conclusions

The interpretations of the results obtained in this study of the effects of Crustastunning apply most directly to the two species used (the crab *Carcinus maenas* and the Norway lobster *Nephrops norvegicus*). However, due to their virtually identical anatomies and physiologies, there is no reason to believe that similar results would not be obtained in other commercially-important UK species such as the edible brown crab, *Cancer pagurus* and the European lobster *Homarus gammarus,* or indeed in other species commonly supplied live to the restaurant trade, such as the American lobster *Homarus americanus* or rock lobster species such as *Palinurus elephas, Panulirus argus* or *Jasus lalandii.* Confirmation of this could only be obtained, however, by directly testing them in a similar manner to that described here.

Acknowledgements