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THE CUTICULAR STRESS DETECTOR (CSD2) OF THE CRAYFISH

I. PHYSIOLOGICAL PROPERTIES

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SUMMARY

1. The cuticular stress detector (CSD2) is coupled to a patch of compliant cuticle in the ischiopodite. It is excited when the compliant cuticle is deformed directly or by deformation of the surrounding stiffer cuticle.

2. Sinusoidal force (1 Hz) directly applied to the compliant cuticle revealed sensory units with thresholds as low as 0·03 mN. The thresholds of most units are higher by up to 40 dB. The stimulus–response curves of the CSD2 units in all preparations have an activity peak between 10 and 30 Hz.

3. In some units (on-units) the spike frequency is high while the force on the cuticle increases. The activity of other units (off-units) declines with rising force. The phase relationship between stimulus and response and the magnitude of the response depend on the exact site of force application.

4. The sensory units of CSD2 can supply the CNS with detailed information about the forces acting on the surrounding cuticle.

INTRODUCTION

Cuticular stress detectors (CSDs) are mechanoreceptors in the legs of decapod crustaceans and were first described by Wales, Clarac, Dando & Laverack (1970) and Wales, Clarac & Laverack (1971). They are chordotonal organs. Moulins & Clarac (1972) showed that the CSD2 of crayfish contain scolopidia with one or two sensory cells embedded in two strands of elongated cells. These strands do not cross a joint and are not connected to a muscle as in other chordotonal organs (Mill, 1976). They are attached to a patch of relatively compliant cuticle in the ischiopodite (CSD2) or basipodite (CSD1) (Fig. 1A,B). Depressing this patch directly or applying force to neighbouring cuticle by moving joints or stretching muscles stimulates the sensory units of CSDs (Clarac, Wales & Laverack, 1971).

This study was limited to CSD2. To obtain quantitative data on the threshold sensitivity of CSD2 and its frequency response, the cuticle near the sense organ was

Key words: Crustacea, proprioceptors, stress detector, cuticle.

deformed by a force modulated sinusoidally. The present experiments on CSD2 physiology allow comparisons with mechanoreceptors in the exoskeleton of other arthropods with a similar function, i.e. the campaniform sensilla of insects and the slit sensilla of spiders (Barth, 1981).

In a second paper (Klärner & Barnes, 1986) the activity of CSD2 during walking and its influence on leg coordination are described.

MATERIALS AND METHODS

Crayfish (*Astacus leptodactylus*) purchased from a commercial supplier (Langbein, Hamburg) were kept in freshwater aquaria at about 13 °C.

Recordings were made from CSD2 units of single walking legs, mainly leg 4, of crayfish weighing approximately 60 g. The leg was amputated at the coxopodite, the meropodite cut off distally, and the nerve exposed by removing the coxopodite and part of the basipodite (Fig. 1A). After mounting the meropodite on a Perspex platform with dental cement, and thereby fixing the mero–ischiopodite joint, the leg was immersed in cooled (11 °C) aerated saline (Dudel & Kuffler, 1961).

The CSD2 was either stimulated with a piezoelectric bender or an electromagnetic vibrator (Ling V 106). The crystal produced the weak stimuli needed to determine thresholds, and the vibrator produced stronger stimuli (>0·6 mN). Both were driven by a function generator (Levell E1. or Exact E1. 119) and could be lowered onto the cuticle with a micromanipulator. A steel pin was glued to the free end of a force transducer (Pixie 8121), which in turn was fixed to the stimulating device (piezoelectric bender or vibrator). This pin was lowered at a right angle and far enough against the cuticular surface that its tip (diameter 0·1 mm) did not lift off the cuticle while a stimulus was applied. Calibration of the force transducer (Pixie) with weights or another force transducer (Grass FT03C) gave 1·1 $mV\,mN^{-1}\,V^{-1}$ source voltage for the piezoelectric bender and 0·68 $mV\,mN^{-1}\,V^{-1}$ source voltage for the vibrator. The force transducer was used to measure the applied forces and to estimate the compliance of the cuticle (Chapman & Duckrow, 1975). Peak-to-peak forces of the stimuli are given.

The response of the sense organ was recorded with suction electrodes in the basipodite (3–4 mm from CSD2) from an axon bundle containing the CSD2 units. After amplification (Isleworth E1. A101) the signals were recorded on magnetic tape. The recordings were analysed on a MINC computer, and peristimulus–time (PST) histograms were plotted. Spike frequency always gives the number of spikes per second averaged over 12 s. This time interval excludes the onset of force exertion on the cuticle and the few seconds needed to adjust the vibrator such that it did not lift off while the stimulus was applied. No adaptation of the sensory units was observed during this 12 s time interval. Mean and standard deviation are given. Circular mean and standard deviation were calculated for the analysis of the CSD2 responses to sinusoidal stimuli (Batschelet, 1965).

RESULTS

Anatomy and cuticle deformation

CSD2 is situated on the ischiopodite near the basipodite joint. The position of the sense organ is marked by a patch of compliant cuticle, which is divided by a protruding part of stiff cuticle (*p*, Fig. 1B). A tongue of stiff cuticle protrudes into the ventral part of the compliant cuticle (*t*, Fig. 1B). The dendrites of the sensory cells are embedded in two tissue strands (Wales *et al.* 1971). Both strands insert on the tongue of stiff cuticle (Fig. 1C). If not stated otherwise, CSD2 was stimulated by moving the distal end of this tongue inwards. The compliance of this area is $1{\cdot}3 \pm 0{\cdot}3\,\mu m\,mN^{-1}$ ($N = 8$) and that of adjacent compliant cuticle is

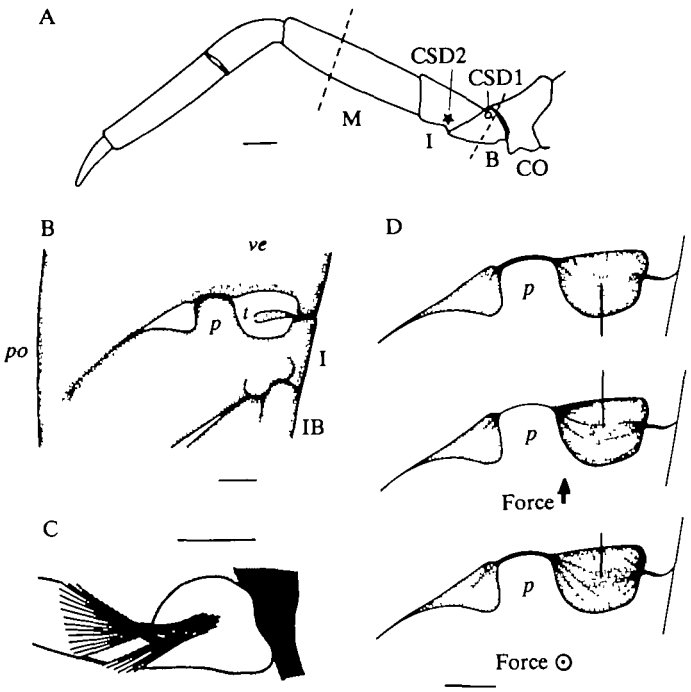

Fig. 1. Anatomy of CSD2 in a crayfish fourth leg. (A) Position of CSD1 (dorso–anterior) and CSD2 (ventro–posterior); M, meropodite; I, ischiopodite; B, basipodite; CO, coxopodite. The dashed lines indicate where the cuticle of the amputated leg was cut prior to recordings of the CSD2 nerve. (B) Ventro–posterior view of the proximal ischiopodite (I). CSD2 lies near the ischio–basipodite (IB) joint. A protruding bit of stiff cuticle (*p*) subdivides the compliant cuticle (not shaded) and a tongue (*t*) projects into the ventral part. *po*, posterior; *ve*, ventral. (C) Interior view of the compliant cuticle. Overlying connective tissue, fixed in alcohol, was removed to expose the tissue strands in which the scolopidia of the sense organ are embedded. (D) Compliant cuticle of CSD2 stippled. The drawings are made to scale while applying a force much larger than that used in the other experiments. Top, no force is exerted on the cuticle; middle, force is applied on the ventral basipodite towards the long axis of the leg; bottom, force is applied perpendicular to the surface of the ischiopodite, about 5 mm from the compliant cuticle. Scale bar, 5 mm in A, 0·5 mm in B, C, D.

$1{\cdot}5 \pm 0{\cdot}4\,\mu m\,mN^{-1}$ ($N = 8$) (stimulus: 10 Hz, 5–8 mN). The proximal end of the tongue is stiffer and has a compliance of $0{\cdot}4 \pm 0{\cdot}2\,\mu m\,mN^{-1}$ ($N = 8$); the stiff cuticle near the joint of the leg is still less compliant ($<0{\cdot}3 \pm 0{\cdot}1\,\mu m\,mN^{-1}$, $N = 8$). The compliance of the cuticular areas tested is approximately linear in the range tested (0·01–12 mN).

The sense organ is sensitive to deformations of the compliant cuticle. These depend on the magnitude, site and direction of the force acting on the cuticle. When force is applied at the insertion of the cuticular tongue, the end protruding into the compliant cuticle moves outwards, when depressed more distally it moves in relative to the cuticular surface. If force is exerted on the ventral basipodite in the direction of the long leg axis, compressing the leg longitudinally, the proximal rim of the compliant cuticle moves distally and the cuticular tongue moves inwards (Fig. 1D). Folds appear on the compliant cuticular patch which are roughly perpendicular to the direction of the applied force. A similar deformation due to similar forces is likely to occur when the leg presses against the ground during the power stroke in walking. Depressing the cuticle of the proximal ischiopodite perpendicular to its surface also deforms the compliant cuticular patch. As indicated by the resulting folds, its deformation differs from that induced by longitudinally applied forces (Fig. 1D).

Excitation of CSD2 units

Response to force exerted on the cuticle

Recordings from the CSD2 nerve show spontaneously active units. Fig. 3A gives the average spontaneous activity of 60 units analysed in 45 preparations; the median is $1{\cdot}1$ spikes s^{-1}. The spike frequency increased when a vibrator depressed the cuticle (Fig. 2). Sometimes, especially with strong stimuli (>2 mN), a second activity peak was apparent when the vibrator moved upwards again and the force exerted decreased. Simultaneous measurements of the applied force showed no distortion of the sinusoidal waveform. It is therefore assumed that the displacement of the vibrator was also undistorted.

In 76 % of the units the spike frequency increased when the cuticle was depressed (on-units), and the spike frequency of 18 % of the units decreased during cuticle depression and increased when the vibrator moved upwards again (off-units). Fig. 2B shows both types in one recording. Only 6 % of the units changed from an on-response near threshold to an off-response to strong stimuli.

If not indicated otherwise, CSD2 was stimulated at the distal end of the tongue projecting into the compliant cuticle (P1, Fig. 4) and the average spike frequency over the stimulus time (12 s) was measured. The sense organ also responded to depression of the surrounding cuticle. For comparison of the sensitivity to force applied at different areas, it was stimulated at two other points, exerting about the same force in all three positions (maximal difference 10 %). The excitation of all on-units was weaker when the proximal (P2), instead of the distal (P1), end of the cuticular tongue was depressed (Fig. 4), while the activity of some off-units was higher. Comparing the phase between stimulus and response there are clear-cut

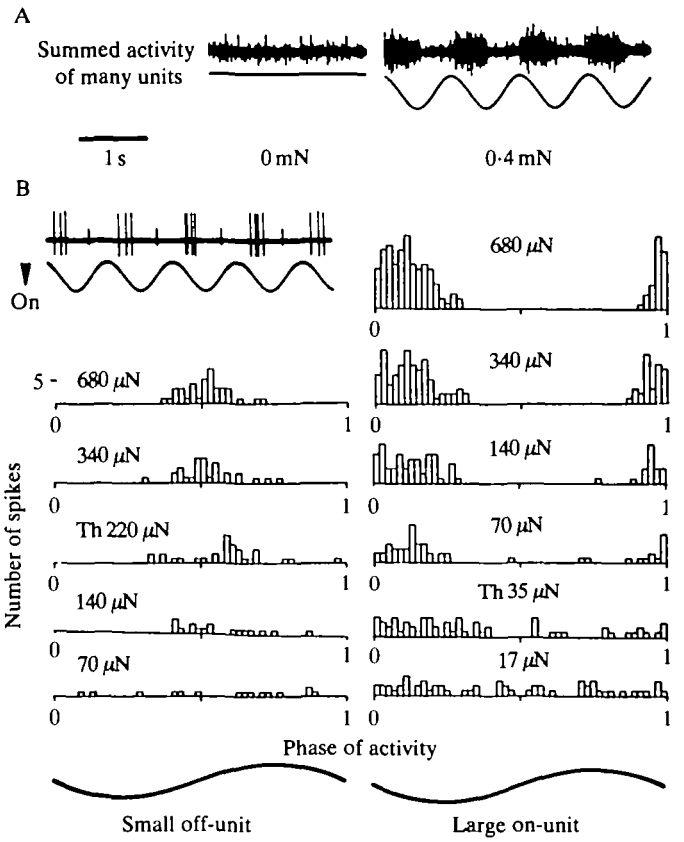

Fig. 2. Recordings from CSD2 units. (A) Spontaneous activity of CSD2 nerve and response to 1 Hz stimulus. (B) Response of small off-unit and larger on-unit to 1 Hz stimulus (0·68 mN). Peristimulus–time histograms (50 stimulus cycles) are given for several stimulus amplitudes including the threshold response (Th).

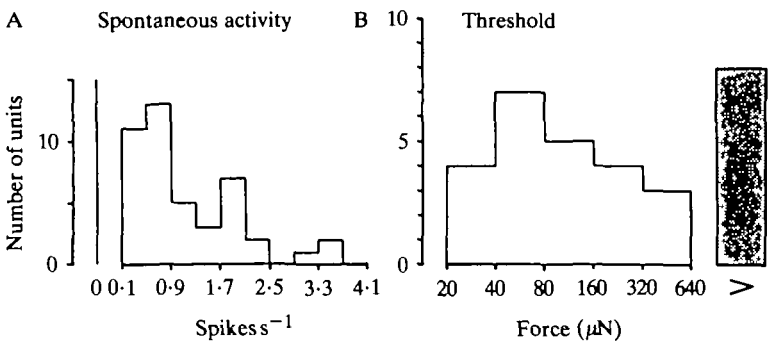

Fig. 3. (A) Spontaneous activity of 60 CSD2 units (45 preparations). (B) Threshold forces of 31 CSD2 units (24 preparations), i.e. applied force at which the peristimulus–time histograms of 12 stimulus cycles were just significantly different from a uniform distribution ($P < 0{\cdot}05$). The right column gives the number of units with thresholds above 0·64 mN. Stimulus frequency, 1 Hz.

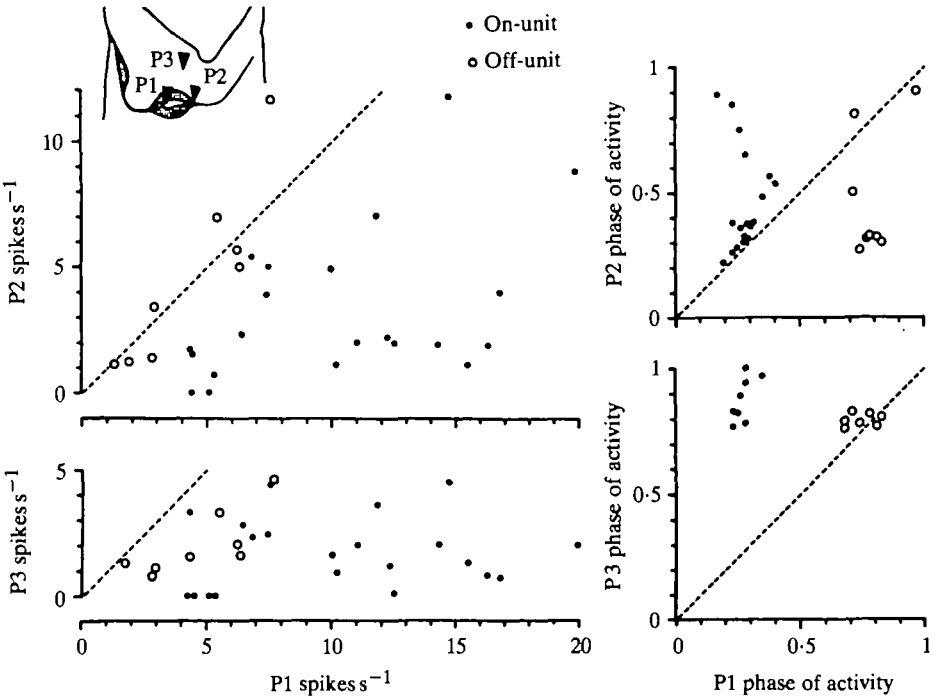

Fig. 4. Response of CSD2 units to stimuli (3 Hz) applied at different positions perpendicular to the cuticle (P1, P2, P3). 0·5 = phase of force maximum, 0 and 1 = phase of force minimum. In recordings of the same unit the forces were approximately the same in all three positions. Equal response to stimuli at two different positions would be along the dashed line.

shifts in a few on-units and relatively many off-units. The variability is large, perhaps because it was difficult to stimulate different legs at exactly corresponding positions. When the stiff cuticle near the ventral basi–ischiopodite joint (P3) was depressed, the response was small compared to that produced by stimulating P1; this applies to all units tested here. There was no clear shift of the phase between stimulus and response for off-units, but the mean activity of on-units was shifted by 0·2–0·5 (1 = 360°) and so became similar to the situation found for off-units.

The complex cuticular structure of CSD2 (Fig. 1) possibly explains (i) the fact that the phase between stimulus and response depends on exactly where the stimulus is applied to the leg, and (ii) why units which respond with a different phase relationship are found for the same stimulus (Fig. 2B). It is likely that the same stimulus deforms the various dendrites of CSD2 differently, and that the deformation depends on the position where the force is applied (see Fig. 1D).

Stimulus–response curves

In these experiments, CSD2 was stimulated by force applied to the distal end of the cuticular tongue (P1, Fig. 4). Fig. 3B shows thresholds of 31 units (1 Hz, sinus). A stimulus was defined as being above threshold if the PST histogram

of 12 stimulus cycles was significantly different from a uniform circular distribution ($P < 0{\cdot}05$, Rayleigh-test) (spontaneous activity would give a uniform circular distribution). The mean spike frequency of all sensory cells increased with stronger stimuli, whereas the slope of this stimulus–response curve varied in different units (Figs 2B; 5A).

The response of sensory cells not only depends on the force applied but also on the stimulus frequency. The activity maximum was in the range 10–30 Hz. Fig. 6 illustrates the variation in the stimulus–response curves with three examples (A–C) and shows the mean response of 30 units (D). The threshold decreases with increasing stimulus frequency up to 30 Hz (Fig. 5B). The mean spike frequency of CSD2 units approximately follows a power function of forcing frequency up to stimulus frequencies of 10 Hz. Fig. 7A demonstrates the variability of the units and Fig. 7B gives the exponent of the power function (i.e. the slope of the linear regression) for 21 on-units and 9 off-units.

The phase between stimulus and response is approximately constant up to 6 Hz stimulus frequency. If 0° is defined as the phase of the force maximum, the mean of the PST histogram is at about 290° (=−70°) for on-units. Stimulus frequencies higher than 6 Hz result in an increase of the phase shift of the response (Fig. 7C). This increase corresponds to a roughly constant delay of about 10 ms, irrespective of the stimulus frequency and is likely to be due to stimulus transduction and impulse conduction. The response of off-units shows a phase shift of about 180° with respect to on-units.

DISCUSSION

Deformation of the cuticle

CSD2 responds to deformation of the nearby cuticle (Clarac *et al.* 1971), which can result from forces acting on the leg during walking (Klärner & Barnes, 1986). When force is exerted on the basipodite in the direction of the long leg axis, compressing the leg longitudinally, the tongue of stiff cuticle which projects into the compliant cuticle of CSD2 moves inwards. Possibly the leg is also compressed and the compliant cuticle deformed in turn in a similar way while the leg is pressing against the ground during walking. When recording from CSD2 units, the natural stimulus was mimicked by a vibrator which depressed the stiff tongue projecting into the compliant cuticle of CSD2.

Thresholds and working range of sensory units

The 60 or so sensory cells of CSD2 (Clarac, 1976) differ in threshold, frequency tuning and phase relationship between stimulus and response. Thresholds as low as 0·03 mN were found for 1 Hz sinusoidal stimuli, but most CSD2 units had higher thresholds. About 40 dB difference between highest and lowest thresholds and about 30 dB dynamic range of single sensory cells give the sense organ a broad working range. Thus, CSD2 is likely to respond both to small deformations of the cuticle

caused by the moving animal as well as to potentially harmful external forces acting on the leg. Together, the CSD2 units can supply the nervous system with a detailed picture of the forces acting on the cuticle of a leg. Therefore CSD2s are likely to

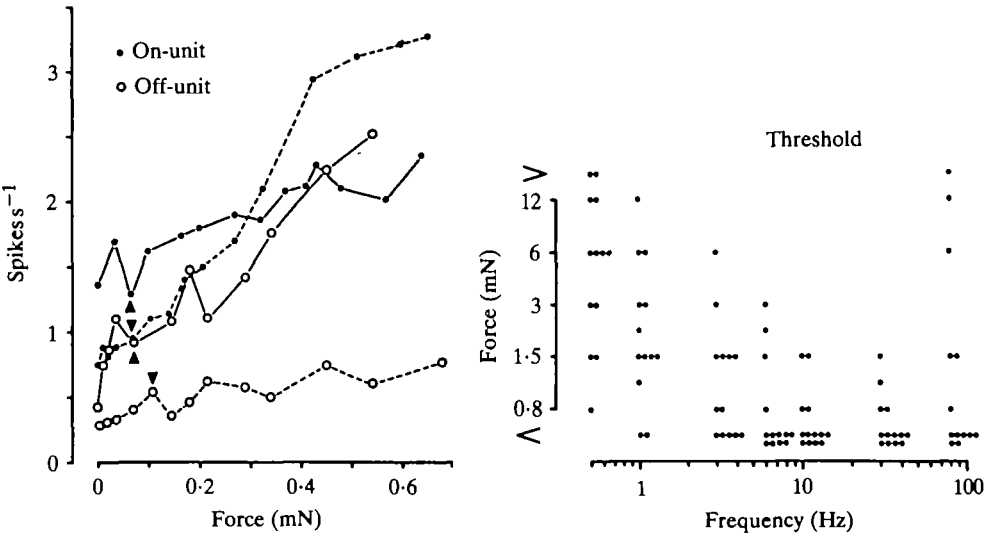

Fig. 5. (A) Stimulus–response curves with thresholds (▼); stimulus frequency, 1 Hz. (B) Thresholds of 13 relatively insensitive units, i.e. peristimulus–time histograms sampled over 12 s of continuous stimulation were just significantly different from a uniform distribution ($P < 0{\cdot}01$).

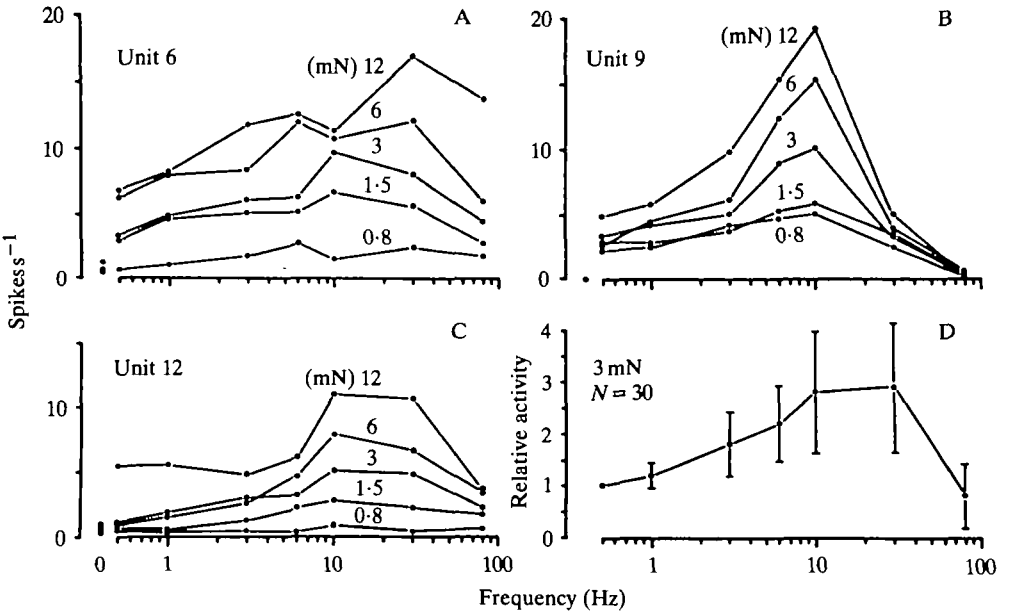

Fig. 6. (A)–(C) Stimulus–response curves of three on-units. (D) Standardized response to 3 mN stimulus. Average spike frequency during the 24 cycles of a 0·5 Hz stimulus = 1, mean and standard deviation for responses of 30 units are given for the other stimulus frequencies.

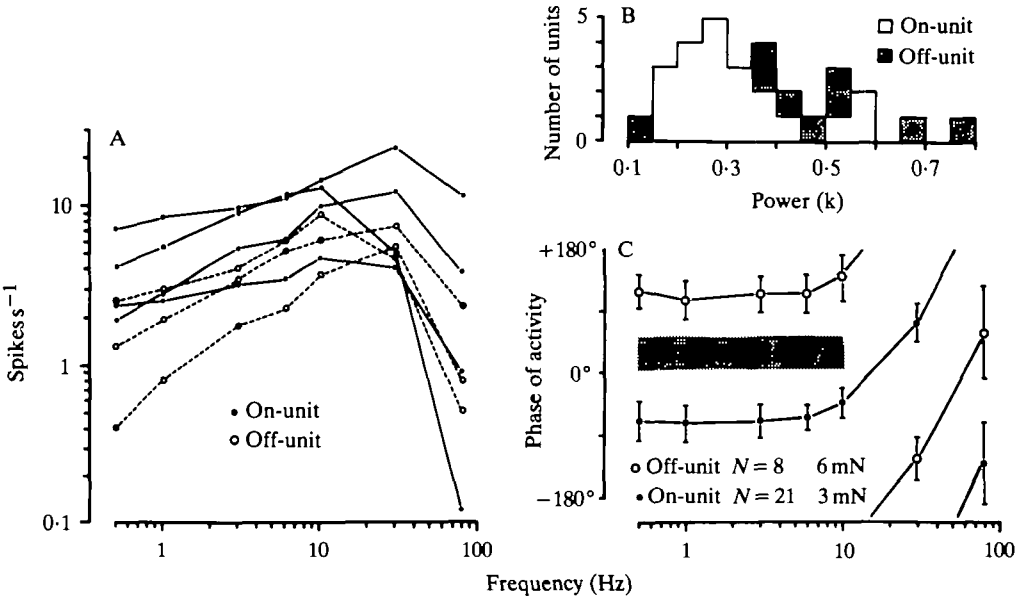

Fig. 7. (A) Stimulus–response curves for on-units (3 mN) and off-units (6 mN). Spikes s^{-1} = average frequency over the 12 s stimulus. (B) Exponents (k) of the power function that approximately describes the relationship between the response and stimulus frequencies up to 10 Hz (means of 2–5 different stimulus amplitudes). (C) Phase shift between stimulus and response for on-units and off-units; values of an s^k transfer function should lie in the stippled area.

monitor loads in the leg exoskeleton so that excessive strains can be avoided and the various gaits adjusted during walking (see Klärner & Barnes, 1986).

CSD2 units respond best to 10–30 Hz stimuli; at higher stimulus frequencies their force sensitivity decreases steeply. That is in contrast to proprioceptors of other arthropods which are also sensitive to cuticle deformation: campaniform sensilla of cockroaches (Chapman, Mosinger & Duckrow, 1979) and slit sensilla of the spider *Cupiennius* (Barth, 1981; Barth & Bohnenberger, 1978; Bohnenberger, 1981) show a high-pass characteristic up to 1 kHz when their displacement or force sensitivity is measured. This difference coincides with the behaviour of the animals. Crayfish move slowly, usually at less than one step s^{-1}; whereas stepping frequencies of 20 Hz are not exceptional for cockroaches (Zill & Moran, 1981) and *Cupiennius* is able to catch cockroaches a few centimetres off in 0·2–0·7 s (Melchers, 1967). Though CSD2 could respond to forces produced during such movements, it would be unable to give detailed information about the time course of the forces. In decapod crustaceans, on the other hand, some mechanoreceptors which are probably sensitive to substrate-borne vibrations do respond to high stimulus frequencies. The displacement threshold of funnel-canal organs (Schmidt & Gnatzy, 1984) on the dactylopodite of *Carcinus maenas* decreases from 1 to 20 Hz and stays approximately constant up to 200 Hz (Barth, 1980), and Barth's myochordotonal organ in the meropodite of *Ocypode* is most sensitive at 1–3 kHz substrate acceleration (Horch, 1971).

Transfer function

This study was not intended to determine the transfer characteristics of CSD2 units. The following remarks, however, seem worthwhile with regard to a comparison with similar receptors.

Up to about 10 Hz, the mean spike frequency of the units varies approximately according to the power of stimulus frequency (exponent k: 0·14–0·75). Similar power functions were found up to stimulus frequencies of 100 Hz for campaniform sensilla (Chapman *et al.* 1979) and slit sense organs (Bohnenberger, 1981). As in CSD2, the mean spike frequency of a single slit on the pretarsus of spider legs does not continue to increase exponentially at stimulus frequencies above 10 Hz (Speck & Barth, 1982). Unlike the other two mechanoreceptors, the response of CSD2 units cannot be described by an s^k transfer function (Bohnenberger, 1981; Chapman *et al.* 1979) because the phase shift between stimulus and response is greater than $k = 90°$ (Fig. 7C). In addition, the phase relationship of stimulus and response is dependent both on the sensory cell studied and on the exact position of the stimulating probe on the cuticle.

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