J. exp. Biol. (1978), 77, 89–106
With 7 figures
Printed in Great Britain

DIRECTIONAL SENSITIVITY IN A CRAYFISH MECHANORECEPTIVE INTERNEURONE: ANALYSIS BY ROOT ABLATION

BY PATRICIA M. FLOOD
Department of Biology, Bryn Mawr College, Bryn Mawr, Pennsylvania 19010

AND LON A. WILKENS
Department of Biology, University of Missouri – St Louis
8001 *Natural Bridge Road, St Louis, Missouri* 63121

(*Received* 27 *February* 1978)

SUMMARY

1. Within the hierarchy of sensory interneurones in the crayfish, individual cells exist which code specifically for directional components of waterborne stimuli. The caudal photoreceptor interneurone (CPR) exhibits this mechanoreceptive response pattern, in addition to its primary light response. However, despite some variability the CPR is bidirectional, responding to water currents in both rostral and caudal directions.

2. The peripheral mechanoreceptive fields providing input to the CPR have been examined, using selective root ablation procedures. Receptors from the exopodite and from the distal portion of the telson are the *primary* sources of input, this being carried by the 2nd and 5th roots respectively of the caudal ganglion. Input from the ipsilateral roots is normally excitatory; input arising contralaterally has an inhibitory influence.

3. Populations of mechanoreceptors presynaptic to the CPR appear to be segregated in terms of the directional information encoded by the CPR. Specifically, ablation of the 2nd ipsilateral root decreases caudal sensitivity predominantly, whereas ablation of the 5th ipsilateral root primarily decreases rostral sensitivity.

4. Therefore, only primary sensory cells which originate from the exopodite, and respond to caudal deflexions of the hair shaft, effectively excite the CPR. Similarly, only the sensory cells originating from the telson which are responsive to anterior deflexions appear to effectively excite the CPR.

INTRODUCTION

The tactile mechanoreceptors on the crayfish telson and thorax are sensitive primarily to movements in the rostrocaudal plane. Movement of the hair shaft is restricted to this plane by exoskeletal articulations, thus producing the directional specificity (Wiese, 1976). Most receptors are dually innervated, one of the two sensory cells being activated by an anterior displacement of the sensillum, the other by a posterior displacement (Mellon, 1963). Recent studies have shown that the crayfish caudal photoreceptor interneurone (Wilkens & Larimer, 1972), and other mechanosensitive interneurones (Wiese, Calabrese & Kennedy, 1976), also respond to displacements of hair sensilla by the water environment, primarily in the rostrocaudal plane. Most of these cells appear to be unidirectionally sensitive, including both primary and higher-order sensory interneurones (Wiese *et al.* 1976).

Directional sensitivity in primary interneurones is apparently the result of a selective pattern of synaptic contact between the interneurone and a population of afferent fibres, all of which respond to the same directional component of the stimulus (Wiese *et al.* 1976). This pattern of connectivity is assumed to begin early during development (Letourneau, 1976*a*), and appears to be complemented by a high level of structural specificity, including a general somatotopic organization of afferent fibres in the ganglionic roots (Letourneau, 1976*b*) and neuropile (Roth, 1976). Directional sensitivity in higher-order interneurones, as described by Wiese *et al.* (1976), is similar to the response pattern characteristic of primary interneurones. Both excitatory and inhibitory polysynaptic inputs have been observed, although neither appears to be required for directional sensitivity *per se*.

In the experiments by Wiese *et al.* (1976), directional stimuli were focused primarily on tactile hairs within the 4th root receptive field on the telson. To fully understand the basis for directional mechanosensitivity, particularly for high-order cells receiving both mono- and polysynaptic sources of input, the entire receptive field must be considered. In the experiments reported here, we have analysed the dynamic mechanosensitive response of a complex multimodal sensory interneurone, the crayfish caudal photoreceptor (CPR). In addition to its primary light sensitive (Prosser, 1934) and pacemaker (Kennedy, 1963) properties, the CPR functions as a unisegmental mechanosensitive interneurone, receiving input from tactile hairs located on the telson and uropods. Its rostrocaudal sensitivity (Wilkens & Larimer, 1972) and involvement in a light avoidance reflex (Welsh, 1934; Harris & Stark, 1974) make the CPR an attractive choice for a study of complex physiological behaviour. Most important for a quantitative analysis of cellular mechanisms, however, is the unique identifiability of this symmetrically paired cell, so that experiments can be conducted on the same cell in successive preparations.

The CPR originates in the 6th abdominal ganglion and has an ipsilateral dendritic tree (Wilkens & Larimer, 1972). The otherwise regular spontaneous discharge of the dark-adapted CPR is modulated by afferent input through each of the five paired sensory roots of the 6th ganglion. Contralateral root input inhibits the CPR while input from the ipsilateral roots produces mixed excitatory and inhibitory effects (Kennedy, 1963; Wilkens & Larimer, 1972; Galeano & Beliveau, 1973).

These experiments were designed to elicit physiologically relevant interneuronal responses from rostrocaudal, water-borne stimuli directed over the entire CPR receptive field on the telson and uropods, and with these appendages positioned in their normal posture. The CRP response has been analysed by selective ablation of 6th ganglion roots during mechanical stimulation. A preliminary report of these experiments has appeared previously (Flood & Wilkens, 1976).

METHODS

The southern crayfish *Procambarus clarkii* (obtained from Monterey Bay Hydroculture Farms, Santa Cruz) was used for these experiments. In the laboratory, these animals were maintained under a controlled diurnal light cycle and kept in aerated spring water. During the experiments, isolated abdomens were mounted on pins, ventral side uppermost, approximately 1 cm above the inclined surface of a recording chamber filled with Sylgard (Dow Corning). The uropods were flared to an extent equivalent to the natural posture observed in unrestrained quiescent animals (angles of 20–28° and 48–54° from the rostrocaudal axis, as measured from the median ridge of the endopodite and exopodite, respectively). The chamber was filled with crayfish saline (van Harreveld, 1936) and all experiments were conducted at room temperature (22 °C).

The ventral nerve cord was exposed in the 4th, 5th and 6th abdominal segments by removal of the soft cuticle, hypodermis and surrounding muscle, blood vessel and intestine. Electrical activity was recorded extracellulary from the CPR in the desheathed 4–5 connective following deafferentation of the 4th and 5th ganglia; all roots of the 6th ganglion were left intact. Conventional a.c. recording techniques were used throughout these experiments. The CPR axon, situated ventromedially in the connective, was identified by its characteristic tonic discharge in response to brief test flashes of light.

Water current stimulus

Periodic bidirectional currents of the saline in the bath were used as the stimulus source. These currents were produced by an apparatus which incorporated an audio speaker mounted securely on one end of the dissecting chamber. A section of the end wall of the chamber equal to the inner cross-sectional area of the bath was cut free and supported by the speaker cone. The continuity of the bath was maintained by a thin flexible rubber membrane. The speaker was driven by a triangular waveform amplified by a programmable d.c. power supply (Kepco, Model BOP-36). With this system, the frequency and amplitude of the periodic driving force could be accurately controlled, thereby permitting the stimulus frequency to be matched empirically to the fundamental harmonic frequency of the recording chamber. At this frequency, a continuous seiche-like oscillation of the saline solution was set up in the bath.

In all experiments, the telson and uropods were positioned opposite the wave generator near the point where the inclined Sylgard floor emerged from the saline solution. By placing the tail appendages near the shallow end of the bath, the receptor hairs were exposed to linear water displacements moving back and forth parallel to the long axis of the animal. Frictional drag from the rising chamber floor in this region of the bath tends to flatten the motion of water particles, an effect analogous to that of waves approaching the shore. Tail position was kept constant from one experiment to the next to minimize stimulus variability.

Water currents in the bath could be observed directly through the dissecting microscope, by watching small particles in suspension moving back and forth along the anteroposterior axis of the abdomen. These currents were monitored by a thin strip of plastic suspended flexibly above the recording chamber by two strain gauges. The

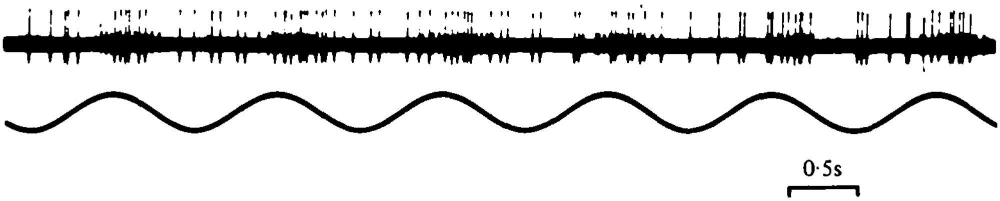


Fig. 1. Electrical activity from the CPR interneurone of the crayfish *Procambarus clarkii*, in response to periodic water current stimulation of the telson and uropods (large spikes, upper trace). The stimulus monitor (lower trace) records linear water displacements near the tail which oscillate sinusoidally. The direction of movement is rostral during positive deflexions (from the lower to upper vertex) and caudal during negative deflexions (from the upper to lower vertex). Similar directions are indicated in Figs. 3–6.

strain gauges served as two arms of a Wheatstone bridge circuit, the output of which was amplified by a d.c.-coupled preamplifier (Tektronix 26A2). This device provided an accurate measure of particle displacement, with the periodic directional saline movements appearing as a regular continuous sine wave as long as the driving frequency was equal to the fundamental periodicity of the recording chamber (1·7–1·8 Hz). A record illustrating CPR activity and the stimulus monitor are presented in Fig. 1.

In each experiment, the plastic sensor was positioned directly above the anus. Although the tail was covered by a layer of water approximately equal in depth to the layer beneath the tail, no attempt was made to quantitatively measure currents beneath the tail. Particle movements, however, appeared similar to those measured above the preparation. Turbulence was not considered to have significantly affected the results, since response patterns were unchanged in their phase relationships, and were qualitatively similar regardless of whether the stimulus movements were large and clearly visible or nearly undetectable by microscopic examination. Due to the amplification factor of large numbers of receptors, interneuronal responses can often be elicited below threshold levels for most primary sensory fibres (personal observation; see also Wiese *et al.* 1976).

Root ablation procedures

Root pairs 1–5 of the 6th abdominal ganglion carry mechanosensory information from receptive fields on the tail appendages as illustrated in Fig. 2 (Calabrese, 1976*a*). To determine the extent to which populations of mechanoreceptors represented by individual roots affected the output of the CPR, each of the ten roots was cut sequentially. During the experiment the preparation was weakly trans-illuminated to facilitate root ablation. However, the CPR was shielded from the light and remained dark-adapted for the duration of the experiment. A constant recovery time of 30 s was allowed between the time a root was cut and the onset of a stimulus trial. Injury discharges recorded in the connectives disappeared during this period of time. Prior to recording any data, an additional 10 s period was allowed following onset of the stimulus, to assure normalization of the saline movements in the bath. Following each root ablation, spike activity was recorded simultaneously from both CPRs for a

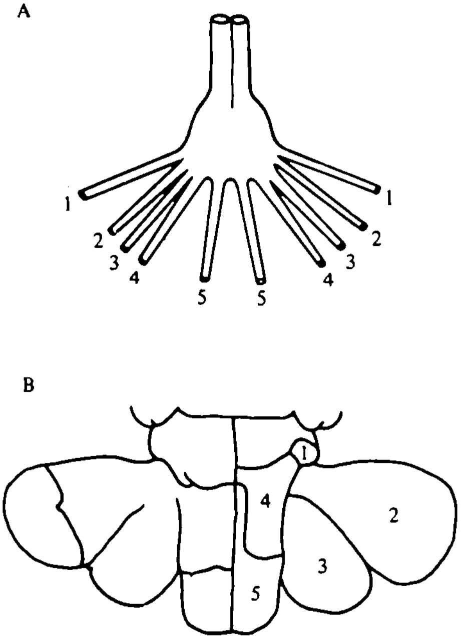


Fig. 2. (A) Diagram of the 6th abdominal ganglion showing the roots containing afferent fibres, i.e. roots 1–5 (as originally numbered by Larimer & Kennedy, 1969). The interganglionic connectives extend upward. (B) Dorsal view of the tail appendages indicating the receptive fields represented by tactile afferent fibres in these five roots (adapted from Calabrese, 1976*a*). The exopodite (area 2) and distal telson segment (area 5) are the major receptive fields for the CPR interneurone.

stimulus train of at least fifty cycles (1 cycle = 360° of sine wave; see Fig. 1) and this activity, along with the output of the strain gauge monitor, was stored on tape using a multichannel FM tape recorder (Sangamo, Sabre II). As a control, fifty cycles of activity were recorded from the intact preparation prior to ablation of any roots. The unstimulated, dark-adapted, endogenous activity of each photoreceptor was also recorded before any of the roots were cut for comparison with the totally deafferented cell at the end of the experiment.

Six different ablation sequences (I–VI) were performed in these experiments. The order in which the roots containing sensory afferents (Fig. 2) were cut is listed in Table 1 for each sequence. In sequence I, for example, the ablation pattern began laterally with root 1 on the right side and progressed toward the midline, followed by a similar order of root ablations on the left. In each experiment complete and sometimes complementary sets of data were obtained for both interneurones. Thus in sequence I the left CPR first lost its input from contralateral receptors, followed by those situated ipsilaterally. At the same time, the right CPR lost ipsilateral, then contralateral input. A similar general pattern was followed in sequences II and III. Ablation sequences IV, V and VI, on the other hand, involve sequential ablation of symmetrical root pairs, a different pair remaining last in each case.

Table 1. *Ablation sequences*

I	II	III	IV	V	VI
1c	5c	1i	1c	1c	1c
2c	4c	2i	1i	1i	1i
3c	3c	3i	5c	5c	5c
4c	2c	4i	5i	5i	5i
5c	1c	5i	3c	2c	2c
1i	5i	5c	3i	2i	2i
2i	4i	4c	4c	4c	3c
3i	3i	3c	4i	4i	3i
4i	2i	2c	2c	3c	4c
5i	1i	1c	2i	3i	4i

In each of the six (I–VI) columns, the sequence in which each of the ten roots was cut is listed in descending order for the left photoreceptor (LCPR) The side on which each root was cut is thus opposite for the RCPR. c = contralateral; i = ipsilateral.

Data analysis

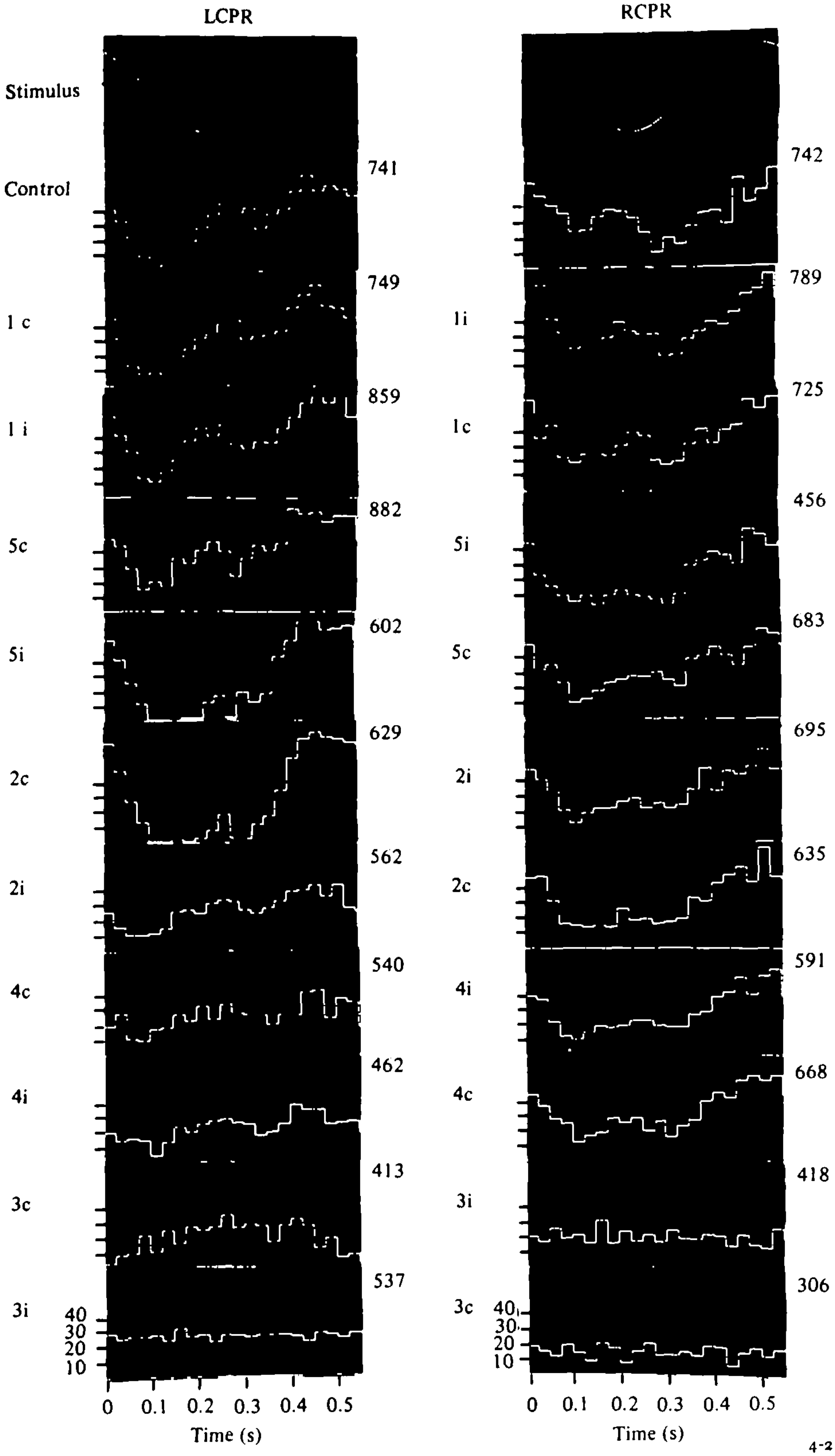
A temporal analysis of the spike trains recorded in response to periodic stimulation, as described above, was subsequently made by computer (PDP-8, Digital Equipment) using a post-stimulus time (PST) histogram program. In all analyses, the computer was set to accumulate data starting with the posteriorly directed stimulus current, i.e. after the positive peak of the sine wave monitor. Due to a small level of baseline noise and the sensitivity of the 'Schmitt trigger', which was set to trigger on negative deflexions from the stimulus monitor, the histograms actually begin 10–15 ms following the positive peak. Each cycle was divided into bins of 25 ms duration. Histograms consisted of spikes accumulated from 45 successive epochs; these were displayed on and filmed from, an oscilloscope. Individual histograms were constructed for the intact (control) preparation and following each of the ten succeeding root cuts.

Several additional control experiments, using the procedures described above were conducted to test for directional responses with respect to receptor alignment, i.e. position of the uropods. Histogram contours were qualitatively similar regardless of whether the uropods were folded, or flared according to their normal posture.

RESULTS

Excluding preliminary trial experiments, these results are based on responses from 17 crayfish (34 CPRs).

Fig. 3. Representative ablation experiment of sequence V (see Table 1), showing the response of the left (LCPR) and right (RCPR) photoreceptors to sequential root cuts in the form of post-stimulus time (PST) histograms. The control histogram (second trace) is from the intact ganglion preparation. The sine wave (top trace) is the stimulus monitor. The number to the left of each histogram indicates the root last cut (i = ipsilateral; c = contralateral). The numbers at the upper right-hand corner of each trace indicate the total spike count (TSC) for that histogram. The effect of the root is measured by comparison of each histogram with the one directly above it. The last histogram in each column represents the spontaneous dark-adapted activity of the completely deafferented preparation.


LCPR
RCPR
Stimulus
Control
741
742
1 c
749
1i
789
1 i
859
1c
725
5c
882
5i
456
5i
602
5c
683
2c
629
2i
695
2i
562
2c
635
4c
540
4i
591
4i
462
4c
668
3c
413
3i
418
3i
537
3c
306
40
30
20
10
0
0.1
0.2
0.3
0.4
0.5
Time (s)

4-2

Control response

The intact tail preparation was used as a control in each experiment. Control CPR responses are usually characterized by two peaks of activity, one associated with the positive and one with the negative vertex of the corresponding sine wave stimulus monitor (Fig. 3). 'Upwards' (positive) on the stimulus trace indicates a rostral current direction; 'downwards' (negative) represents a caudal direction. In most experiments, one of the two activity peaks was predominant, although in a few instances both peaks appeared equal. The predominance of a single activity peak indicates maximal sensitivity for the corresponding rostral or caudal direction of flow. The presence of the second peak, however, represents a departure from the unidirectional sensitivity characteristic of other interneurones (Wiese *et al.* 1976). Surprisingly, the peak directional sensitivity varies from rostral to caudal in CPRs from different animals, in some cases even between the right and left cells in the same animal. This is illustrated in Fig. 4, where the maximum sensitivity of the LCPR corresponds to the negative vertex while the RCPR is most sensitive relative to the positive vertex. Since histogram contours appeared stable in control experiments testing the effect of mounting the tail appendages in various positions (see Methods), response variability is not believed to result from experimental differences. Nevertheless, special care was taken to ensure that the position of the abdomen and the saline level were kept constant in all experiments. Thus, although directional sensitivity is evident in most CPRs (as reported previously; Wilkens & Larimer, 1972), there is no absolute directional preference common to all animals (or CPRs).

The control results from each of the experiments were combined to determine the overall directional sensitivity of the CPR interneurone. Fig. 6 is a plot of these combined CPR responses, and shows the numerically averaged controls (triangles). For easier comparison of the activity peaks, the data has been plotted starting with the 5th bin. The bidirectional sensitivity of the CPR is clearly indicated, though with an apparent slight preference for the rostral current direction. Whether this difference in magnitude of the two activity peaks is significant, however, is not known. The relatively large standard errors result from the inherent directional variability of this preparation (cf. S.E. near peaks and troughs) as well as from possible experimental error in three of the experiments, the data from which is included in the means. Activity peaks in these three experiments appeared to be shifted to the right of the stimulus monitor vertices by 70–100 ms (3–4 bins). In all other experiments, the correspondence between the response peaks and the vertices of the stimulus monitor was close and invariant.

Effects of root ablation

The relative contribution of each afferent root to the response pattern of the CPR was determined by two methods of analysis. First, each time a root was cut, regardless of its order in the ablation sequence, the percentage change in total spike count (TSC) for the 45 epochs (i.e. total spikes per histogram) was calculated by comparison with the preceding TSC. The change in activity was either positive or negative depending on the relative efficacy of excitatory and/or inhibitory afference contained within the cut root. Secondly, ablation sequences were constructed so that each of the five roots

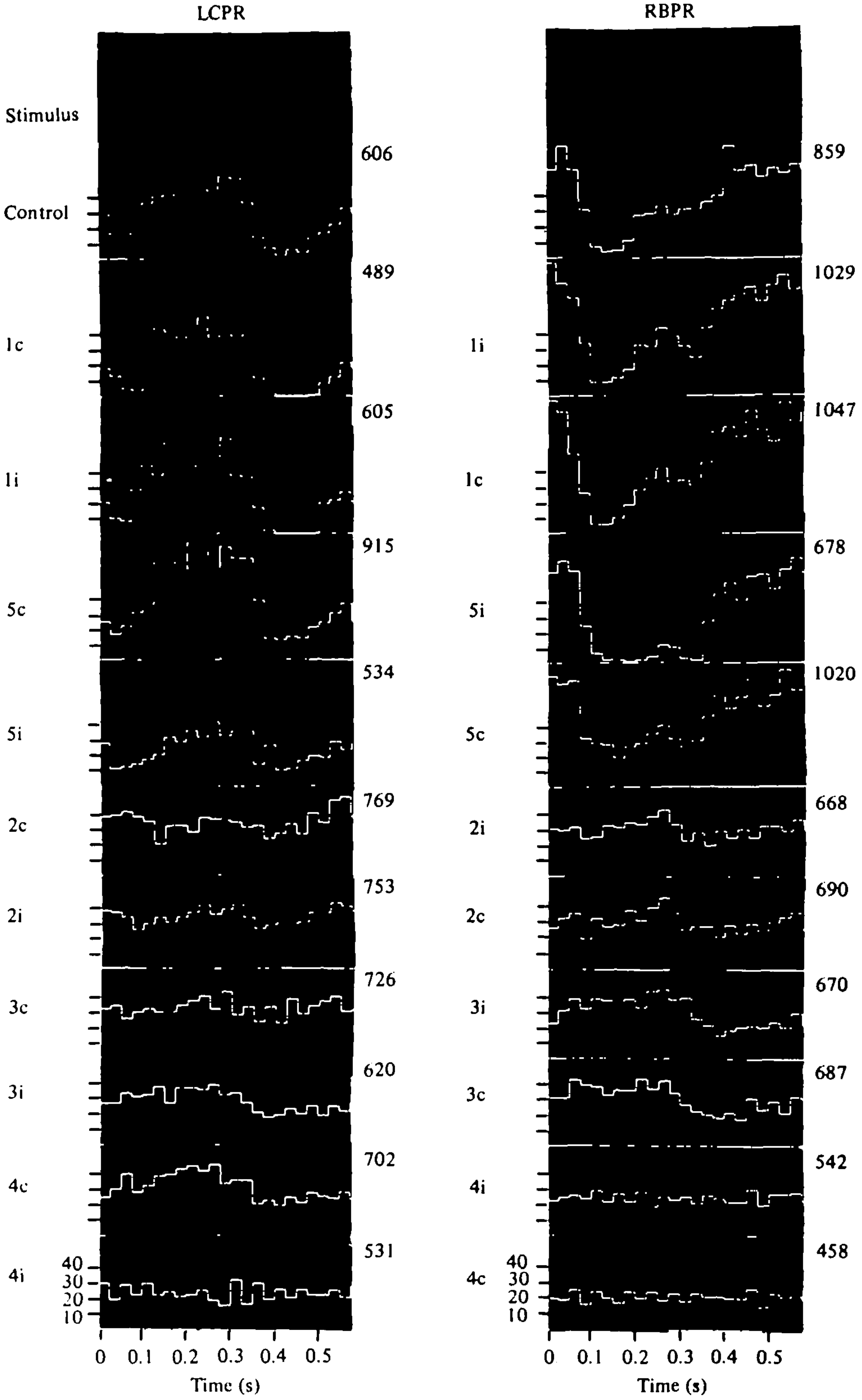


Fig. 4. Representative ablation experiment of sequence VI (refer to Fig. 3 for explanation).

would be the only remaining root in at least several experiments (see Table 1), thereby allowing that root to demonstrate its singular effect on the CPR response. Analysis of histogram plots indicated two basic response patterns as a result of root ablation, either a pronounced decrease of activity associated with one or the other of the two peaks, or a tonic potentiation or inhibition of overall spike discharge. Three of the six sequences are represented in Figs. 3–5.

Root 1. No consistent positive or negative change resulted from cutting either of the first roots, although the TSC varied by as much as 15% in eight of the CPRs tested. The change in the CPR response, whether appreciable or not, was always a general increase or decrease in activity.

Root 2. The 2nd *ipsilateral* root contributes consistently to the phasic response of the CPR. Each time this root was cut, the total spike output of the CPR decreased dramatically (28·5% ± 2·8*), with loss in activity represented predominantly by the same peak in each experiment. Specifically, this loss of activity corresponded with the rostral stimulus direction designated peak β (Fig. 6), i.e. the peak interrupted at the margins in Figs. 3–5. The only exception in these experiments is shown in Fig. 5 (RCPR) where section of ipsilateral root 2 affects primarily the α peak (cf. 2nd and 3rd histogram from bottom). To determine the relative directional effect of the ipsilateral 2nd root quantitatively, the activity change for peak β was determined by comparing the mean amplitude of bins 1, 2, 20, 21 and 22 from all 34 CPRs, before and after ablation of ipsilateral root 2. The amplitude of peak β decreased by an average of 53·4% ± 4·4, indicating that ipsilateral root 2 contributes significantly to this portion of the directional response. Phasic activity correlated with the caudal stimulus direction (referred to as peak α) was also affected by cutting the second ipsilateral root. This peak exhibited a small but fairly consistent increase in activity (mean amplitude change was 4·8% ± 4·6), indicating a slight inhibitory influence in this root also.

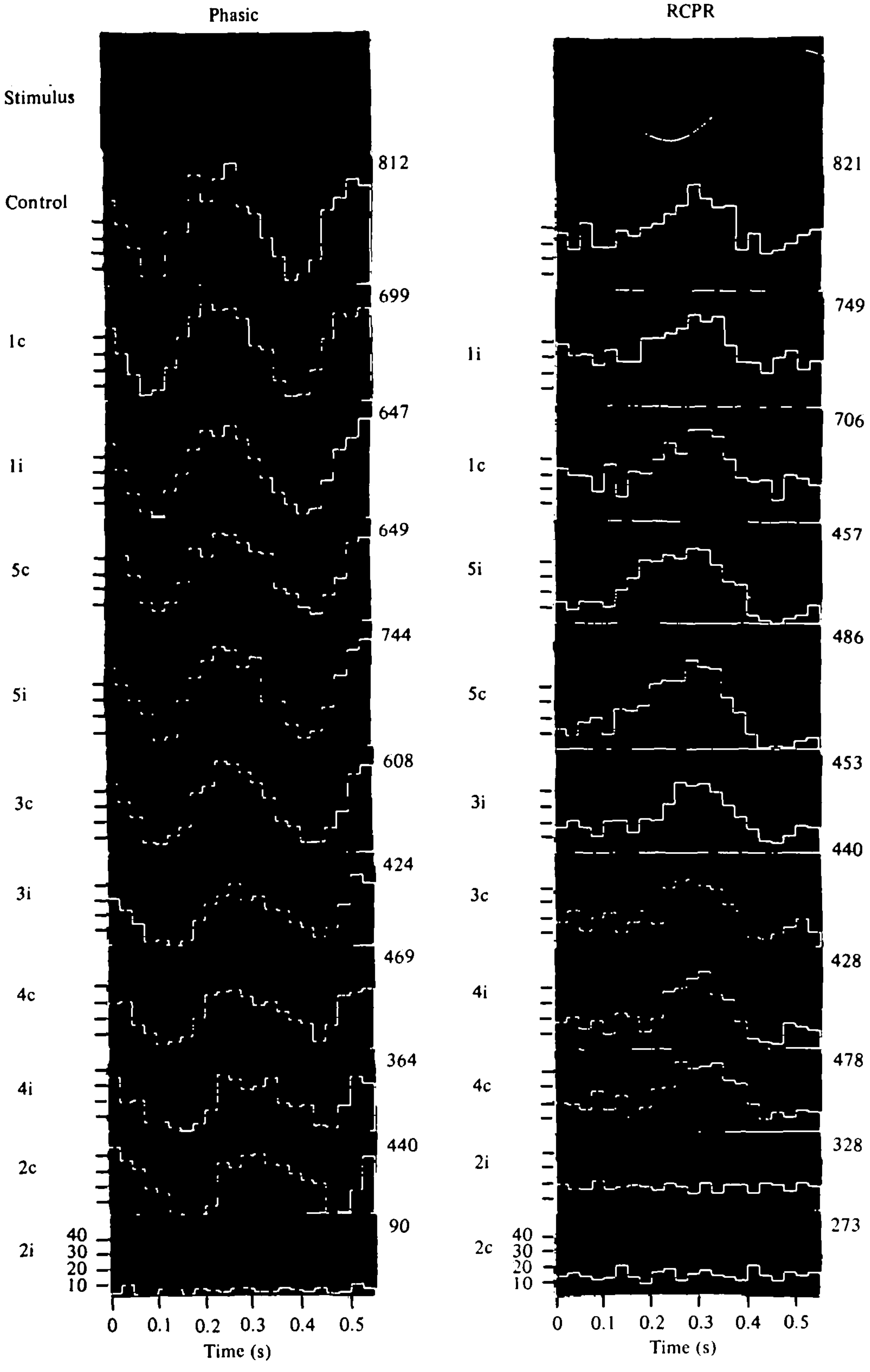
Ablation of the *contralateral* 2nd root, on the other hand, usually resulted in a significant increase in overall activity, as evidenced by an average increase of 19·7% ± 6·6 in the TSC. In several experiments the increase in activity seemed to be correlated with the β-peak, but this effect was not consistent.

Root 3. Loss of input from the 3rd *ipsilateral* root results in only a moderate decrease in the TSC (9·1% ± 2·6). The basis of this decrease appears to be a somewhat unpredictable change in the response of the CPR, where the activity loss occurs in either of the two activity peaks, but not both. This is illustrated in Fig. 3 where the 3rd roots were the last to be cut experimentally. With only the 3rd ipsilateral root intact, the phasic activity in the left CPR corresponds to the α-peak, whereas in the RCPR the remaining phasic activity occurs in the region of the β-peak.

Section of the 3rd *contralateral* root produced an average decrease of 4·9% ± 3·3 in the TSC despite the fact that in half of the experiments, a slight increase in activity, as anticipated, occurred. Overall, the contralateral 3rd root appears to have little influence on the response pattern of the CPR interneurone.

* Standard error of the mean.

Fig. 5. Representative ablation experiment of sequence IV (refer to Fig. 3 for explanation). Only the RCPR activity was analysed in this experiment. A set of histograms from a phasic fibre adjacent to the LCPR are shown in the left-hand sequence.


Phasic
RCPR
Stimulus
Control
812
821
1c
699
1i
749
1i
647
1c
706
5c
649
5i
457
5i
744
5c
486
3c
608
3i
453
3i
424
3c
440
4c
469
4i
428
4i
364
4c
478
2c
440
2i
328
2i
90
2c
273
40
30
20
10
0
0.1
0.2
0.3
0.4
0.5
Time (s)
Time (s)

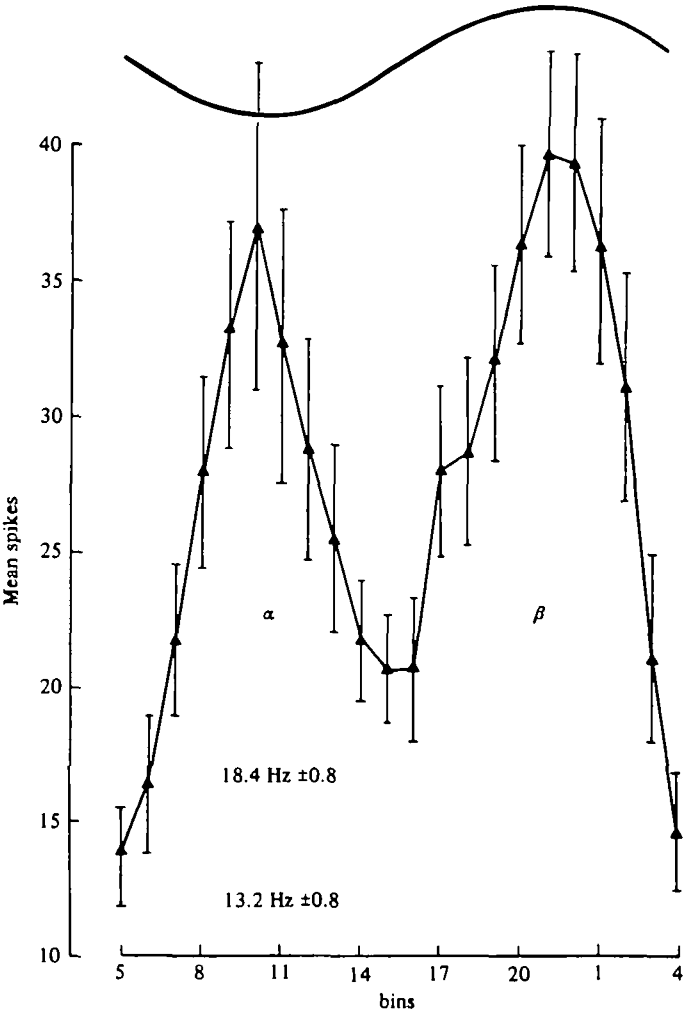


Fig. 6. Response of the CPR interneurone to periodic directional stimulation of telson and uropod mechanoreceptors. The curve represents the mean response from intact (control) preparations. Each data point (triangles) represents the mean number of spikes per bin (± S.E.) for all of the 34 interneurones tested. Data from the first four bins has been shifted to the right-hand side to facilitate comparison of activity peaks; thus bins (25 ms each) are numbered on the abscissa beginning with bin 5. Two peaks of activity (peak α and peak β) occur during the stimulus cycle (sine wave insert); each corresponds closely with a change in the stimulus direction (lower and upper vertex, respectively). The mean unstimulated endogenous rates of the dark-adapted CPRs are indicated for the intact (18·4 Hz ± 0·8) and totally deafferented (13·2 Hz ± 0·8) ganglion preparations, for comparison with tactile-evoked activity.

Root 4. *Ipsilateral* root four mediates a moderate but consistent excitatory input, as evidenced by an average decrease of 8·7 % ± 2·6 in the TSC following root section. This decrease in activity is specific for peak α (Fig. 4).

Following ablation of the *contralateral* 4th root, exactly half of the cells exhibited a small decrease in activity, the other half a slight increase. This is borne out by a mean increase of 0·1 % ± 2·3 in the TSC. Thus the contralateral 4th root appears to have very little effect on the CPR.

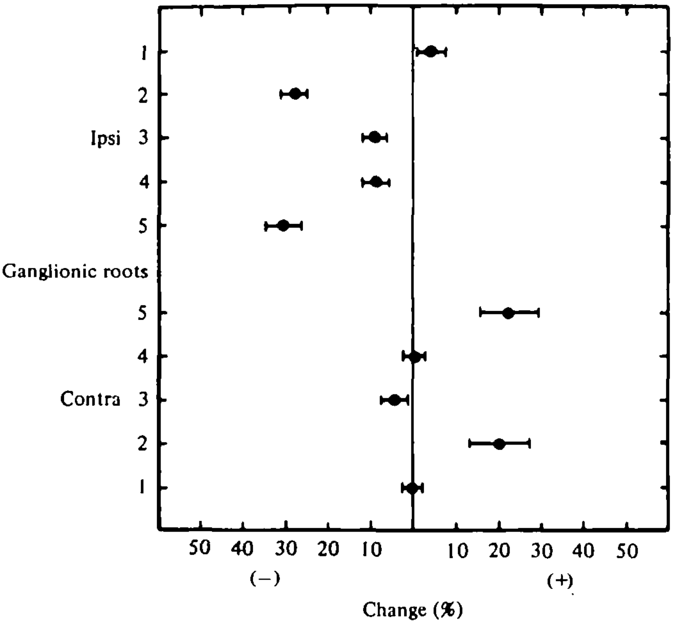


Fig. 7. Summary of the effects of cutting individual roots. The mean percent change and standard error in CPR total spike count (TSC) after cutting each root are plotted irrespective of the ablation sequence. Sample size: $n = 34$ photoreceptors.

Root 5. Ablation of the *ipsilateral* 5th root effected a more profound change in CPR activity than any other single sensory root. An average decrease in the TSC of 31·3 % ± 3·9 following ablation indicates a normally strong excitatory input via this root which is reflected in both components of the CPR's phasic output, although to varying extents. In each CPR whose control histogram included peak α (only 3 controls in 34 interneurones did not), this directional component was reduced by at least 50 %, the mean decrease in amplitude being 57·3 % ± 3·7. The β-peak was reduced significantly (a mean decrease of 19·2 % ± 2·6) in twenty-seven of the CPRs tested. In the other seven CPRs, peak β was relatively unaffected. Since in the majority of experiments cutting ipsilateral root 5 altered both peaks, it appears that the 5th root carries tactile information for both stimulus directions. Tactile input from rostrally sensitive fibres influences the CPR approximately three times more strongly than input from caudally sensitive units, based on the relative α- and β-effects.

The *contralateral* 5th root was observed to exert a consistently inhibitory and phasically non-specific effect on the output of the CPR (Fig. 3). The mean increase in the TSC resulting from ablation of this root was 21·9 % ± 6·1, the most effective source of inhibition among all the contralateral roots.

A summary of the effects of cutting each of the ten sensory roots is presented in Fig. 7. All of the data collected for a particular root are incorporated, regardless of the order in the ablation sequence in which it was cut. Clearly, ipsilateral roots 2 and 5 are the primary sources of excitation while the corresponding contralateral roots are the most inhibitory in their effect on CPR activity. A similar analysis of the data was made in which activity changes were averaged only when a particular root was the first or the last root to be sectioned in various experiments. These results, demon-

Table 2. *Mean discharge rates (Hz) and standard errors (n = 7), during mechanical stimulation, for CPRs with all ganglionic roots intact (A), after unilateral root section (B), and following subsequent total deafferentation (C)*

	Contralateral	Ipsilateral
(A) Ganglion roots intact	20·7 ± 2·5	29·7 ± 6·4
(B) Unilateral deafferentation	32·6 ± 5·3	8·6 ± 1·9
(C) Ganglion completely deafferented	10·3 ± 2·2	13·7 ± 2·3

The left-hand column gives the data for the CPR group which, in (B), had been isolated from all contralateral root input; the right-hand column shows the data for CPR's isolated initially (in B) from all ipsilateral root input. The two CPR groups represent bilateral pairs from the same seven animals.

strating singular root input, were qualitatively identical to those illustrated in Fig. 7, although standard errors were larger due to the smaller sample size.

After the first five root cuts in seven experiments involving sequences I, II or III, the 6th ganglion was always totally deafferented on one side and intact with respect to the symmetrical roots contralaterally. Replicate experiments exist, therefore, in which one CPR receives its full complement of ipsilateral input in the absence of contralateral influences. The reverse situation occurs for the corresponding CPR in the opposite connective. The average spike output of the unilaterally isolated CPR during periodic mechanical stimulation in these seven experiments has been calculated for comparison with both the control (intact) rates and the spontaneous discharge from CPR preparations deafferented bilaterally. These results are presented in Table 2. After the loss of contralateral afferent input there was a 57 % average increase in total spike activity, whereas following ipsilateral deafferentation there was a 71 % average decrease. After total deafferentation of the CPR preparations innervated only ipsilaterally, there was again a large average decrease (68 %) in the rate activity following the loss of ipsilateral input. Similarly, there was a 59 % average increase after total deafferentation of the CPRs receiving only contralateral input. Thus, in the presence of periodic mechanical stimulation, the ipsilateral roots as a group mediate excitation. With the loss of ipsilateral input, either in the presence or absence of contralateral input, there is a consistent reduction in CPR output (−71 % and −68 %, respectively). The opposite effect occurs following the loss of contralateral input; CPR output is consistently increased by (57 % to 59 %) regardless of the presence or absence of excitatory ipsilateral input. Contralateral roots therefore collectively mediate inhibition.

Related bidirectionally sensitive interneurones

In the course of these experiments, two additional fibres highly sensitive to water current stimulation were routinely encountered during the process of isolating the photoreceptor interneurone. Each of the two fibres was quiescent but responded to periodic water currents with biphasic firing patterns similar to that of the CPR. These cells also retained a significant portion of their initial spiking pattern until section of the 2nd ipsilateral root, suggesting that both cells shared the same source of afferent input as the CPR, i.e. tactile hairs on the surface of the exopodite. For comparison, the output of one of these cells was analysed by the same method used for the CPR (Fig. 5, phasic). These results not only confirmed the fact that the biphasic pattern of the response was mediated predominately via ipsilateral root 2, but also showed that an overall excitatory or inhibitory effect was exerted by each of the other nine roots, thus indicating that these cells are interneurones and not sensory cells extending into the connectives. It is interesting to note that ipsilateral root 2 alone can mediate a completely biphasic response in these fibres, although this never occurs in the CPR. As observed earlier (Wilkens & Larimer, 1976), the CPR is only one of an interrelated class of mechanosensory interneurones.

DISCUSSION

Coding of mechanosensory input

In a previous study (Wilkens & Larimer, 1972), the crayfish caudal photoreceptor was shown to be a directionally sensitive mechanoreceptor interneurone, in addition to its light-related physiological behaviour. The results of this study characterize the CPR as a bidirectionally sensitive cell. Of the 34 cells analysed, all but eight had two distinct sensitivity peaks; in four instances the CPR displayed equal sensitivity to both directions. Bidirectional sensitivity is also clearly indicated in the averaged response profile and cannot be related directly to the pacemaker activity of the CPR, since both peaks are substantially above the rate of spontaneous activity observed in either the quiescent or deafferented condition (Fig. 6).

Both peaks of CPR activity correspond consistently with the vertices of the stimulus sine wave monitor. This relationship is also fairly representative of mechanoreceptor activity since, at a frequency of 1·7 Hz, the phase differences between the CPR and primary receptors would be small (2–3 % max.) based on estimates of conduction velocity (Wiese, 1976) and synaptic delay. Thus, both the mechanoreceptors and the CPR appear to respond maximally when the water stimulus is maximum in terms of horizontal displacement. If it is assumed that the mechanoreceptor hair moves in phase with the stimulus, peak activity also occurs as the receptors reach maximum deflexion. Whether sensitivity is to stimulus displacement or acceleration of the stimulus current cannot be determined from these experiments since acceleration is maximum when water displacement is greatest.

A similar phase relationship, for sinusoidal stimuli at a frequency of 5 Hz, has been reported in crayfish for both mechanoreceptors (Wiese, 1976) and sensory interneurones (Wiese *et al.* 1976). However, a quantitative temporal analysis was not made and considerable phase variation appears to exist. Phase variability in mechanoreceptors can be pronounced at low frequencies (0·2–1·0 Hz) for threshold displacements, or it can occur at higher frequencies due to conduction 'dead-time' lags (Wiese, 1976).

The above mechanoreceptor response characteristics were based on results obtained from hairs rigidly coupled to the stimulator by means of a wire loop; no data were presented concerning phase relations using natural coupling via the water medium. A more precise determination of CPR phase relationships will therefore await further experimentation, including a quantitative temporal analysis of mechanoreceptor activity and controls for possible differences between rigidly coupled and naturally coupled stimulus presentations.

The present experiments have demonstrated that directional sensitivity is variable in different CPRs, but that the phase of the response is relatively constant. The directional variability does not appear to have been the result of experimental differences, since procedures were kept constant and the results of control studies did not reflect major changes due to tail position, size, etc. Both response variability and bidirectional sensitivity, the latter being unique with respect to previously described unidirectional cells (Wiese *et al.* 1976), may be inherent in the structuring of the afferent hierarchy centrally. That the CPR is multimodal, and receives a significant level of indirect inhibitory input, indicates that this cell is functionally complex and occupies a high-order position in the sensory system. Response variability may be characteristic of higher-level cells with a greater proportion of polysynaptic input. Also, if anterior neural centres respond to numerous mechanosensitive interneurones ascending as an integrated system, rather than to individual cells, absolute directional specificity may not be as important in cells considered apart from a larger network.

Organization of CPR input

The results obtained through selective root ablations outline a fairly constant pattern of afferent input from the peripheral receptors. Consistently, roots 2 and 5 mediate the largest excitatory (ipsilateral) and inhibitory (contralateral) effects (Fig. 7). Interestingly, bilaterally equivalent populations of receptors are the major source of opposing influences on the same cell.

A second consistent pattern of afferent input has emerged with respect to the directional components of the CPR response. Phasic directional activity in the CPR is specifically related not only to well-defined peripheral receptive fields, but to specific sets of directional sensory cells. For example, the loss of exopodite (2nd root) input to the ipsilateral CPR results in a marked decrease in the phasic response to tailward components of the stimulus cycle, but does not significantly affect the remaining headward response activity. A similar relationship occurs with respect to the 5th root receptive field on the distal portion of the telson, except that these receptors elicit phasic activity in response to headward stimulus directions. The 4th root receptors, located proximally on the telson, also contribute to the formation of the rostral peak, although to a lesser extent.

Directional input to the CPR appears to be specified according to the spatial distribution of receptors on the tail. Only caudally sensitive receptors from the exopodite contribute significantly to the excitatory synaptic input of the CPR; similarly, the rostrally sensitive sensory cells from the telson receptors appear to influence the CPR preferentially. A similar pattern has been described for unidirectionally sensitive interneurones (Wiese *et al.* 1976), where mechanoreceptor cells sensitive exclusively to the same stimulus direction converge on the postsynaptic cell. In the CPR, this pattern of convergence incorporates two distinct regions of opposite polarity involving ipsilateral excitatory input.

Mechanosensory input to the CPR from the 1st, 3rd and 4th roots is generally of lesser significance. Other interneurones undoubtedly exist which reflect a greater influence from the receptive fields represented in these roots. It should be noted, however, that the effect of a given root on CPR activity may vary according to which other roots were intact at the time of ablation, a fact which may in part explain the variability encountered.

Relative effects of inhibitory–excitatory input

Differences exist in the literature with respect to CPR inhibition mediated ipsilaterally versus contralaterally, which arise from the use of different experimental techniques. Galeano & Beliveau (1973) conclude that the overall effect of mechanoreceptive input on the CPR from either side is negative, although inhibition is stronger from the contralateral roots. Unlike our results, those of Galeano & Beliveau (1973) were based primarily on the effects of unilateral root section on spontaneous CPR activity in the unstimulated tail preparation. Hermann & Olsen (1968), on the other hand, report that mechanical stimulation of the peripheral receptors produces a greater inhibitory effect ipsilaterally, although they reported considerable variation and also did not make a systematic study of the roots. Our results, summarized in Fig. 7 and Table 2, support the view (Kennedy, 1963; Wilkens & Larimer, 1972) that contralateral roots mediate negative input while ipsilateral roots exert a predominantly positive influence. The present techniques, however, do not provide the resolution needed to separate completely the mixed excitatory–inhibitory effects known to arise via ipsilateral pathways. A more thorough study of inhibition has been undertaken (Marzelli & Wilkens, 1976), in which the interneurones which mediate negative input from both contralateral and ipsilateral roots are described. A complete description of these pathways will be presented in a subsequent paper.

The temporal expression of input from positive and negative sources is dependent on the central pathways involved. For example, direct monosynaptic input from ipsilateral root afferents, recorded intracellularly in the ganglion (Wilkens & Larimer, 1972), will have short-latency effects on the CPR. Contralateral input, which must be transmitted polysynaptically since primary afferents do not cross the ganglionic midline (Calabrese, 1976*b*) and since CPR dendrites are restricted to their hemiganglion, will encounter longer delay intervals. As a consequence of this delay, contralateral inhibitory input will in general be less accurate for precise temporal patterning, a feature perhaps correlated with the non-phasic expression of inhibition from contralateral roots as observed in the present study. Since ipsilateral inhibition is also polysynaptic (Marzelli & Wilkens, 1976), its effect will also be delayed; however, the effects of excitation and inhibition mediated ipsilaterally could not be separated by the experimental procedures used here. Thus, although the CPR is biphasic at 1·8 Hz, its sensitivity and phase, relative to stimuli over a range of frequencies, will probably vary based on the known circuitry and complex integrative mechanisms carred out centrally.

The authors would like to thank Dr M. Mote for the use of his PDP-8 computer, and Ken Black and Jim Struthers for computer assistance. A preliminary review of the manuscript by Dr J. L. Larimer is also appreciated.

This research was supported by National Institutes of Health grants NS11751 and NS12971 and is a publication (No. 82) of the Tallahassee, Sopchoppy and Gulf Coast Marine Biological Association.

REFERENCES

CALABRESE, R. (1976*a*). Crayfish mechanoreceptive interneurons. I. The nature of ipsilateral excitatory inputs. *J. comp. Physiol.* **105**, 83–102.

CALABRESE, R. (1976*b*). Crayfish mechanoreceptive interneurons. II. Bilateral interactions and inhibition. *J. comp. Physiol.* **105**, 103–114.

FLOOD, P. M. & WILKENS, L. A. (1976). Somatosensory integration in a crayfish interneuron: analysis by root ablation. *Amer. Zool.* **16**, 179.

GALEANO, C. & BELIVEAU, S. (1973). Mechanoreceptor and photoreceptor tonic integration in the crayfish. *Can. J. Physiol. Pharmacol.* **15**, 949–958.

HARRIS, D. A. & STARK, L. (1974). A quantitative approach to habituation and to evaluation of the effects of noisy neural pathways in crayfish system. *Inter. J. Neuroscience* **6**, 181–193.

HERMANN, H. T. & OLSEN, R. E. (1968). Afferent stochastic modulation of crayfish caudal photoreceptor units. *J. gen. Physiol.* **51**, 534–551.

KENNEDY, D. (1963). Physiology of photoreceptor neurons in the abdominal nerve cord of the crayfish. *J. gen. Physiol.* **46**, 551–572.

LARIMER, J. L. & KENNEDY, D. (1969). Innervation pattern of fast and slow muscle in the uropods of crayfish. *J. exp. Biol.* **51**, 119–133.

LETOURNEAU, J. G. (1976*a*). Addition of sensory structures and associated neurons to the crayfish telson during development. *J. comp. Physiol.* **110**, 13–23.

LETOURNEAU, J. G. (1976*b*). Somatotopic organization of afferent axons in peripheral nerves. *J. comp. Physiol.* **110**, 25–32.

MARZELLI, G. A. & WILKENS, L. A. (1976). Central inhibitory fibers in the crayfish abdomen: effects on a sensory interneuron. *Amer. Zool.* **16**, 178.

MELLON, DEF. (1963). Electrical responses from dually innervated tactile receptors on the thorax of the crayfish. *J. exp. Biol.* **40**, 137–148.

PROSSER, C. L. (1934). Action potentials in the nervous system of the crayfish. II. Response to illumination of the eye and caudal ganglion. *J. Cell. Comp. Physiol.* **4**, 363–377.

ROTH, R. L. (1976). Topographic organization of primary afferents to the sixth abdominal ganglion of the crayfish. *Neurosci. Abst.* **2**, 183.

VAN HARREVELD, A. (1936). A physiological solution for freshwater crustacea. *Proc. Soc. exp. Biol. Med.* **34**, 428–432.

WELSH, J. B. (1934). The photoreceptor and response of the crayfish to light. *J. Cell. Comp. Physiol.* **4**, 379–388.

WIESE, K. (1976). Mechanoreceptors for near-field water displacements in crayfish. *J. Neurophysiol.* **39**, 816–833.

WIESE, K., CALABRESE, R. L. & KENNEDY, D. (1976). Integration of directional mechanosensory input by crayfish interneurons. *J. Neurophysiol.* **39**, 834–843.

WILKENS, L. A. & LARIMER, J. L. (1972). The CNS photoreceptor of crayfish: morphology and synaptic activity. *J. comp. Physiol.* **80**, 389–407.

WILKENS, L. A. & LARIMER, J. L. (1976). Photosensitivity in the sixth abdominal ganglion of decapod crustaceans: a comparative study. *J. comp. Physiol.* **106**, 69–75.