

FEEDBACK FROM MOTOR NEURONES TO PACEMAKER NEURONES IN LOBSTER CARDIAC GANGLION CONTRIBUTES TO REGULATION OF BURST FREQUENCY

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Accepted 18 August 1988

Summary

The crustacean cardiac ganglion has traditionally been viewed as a two-layered system in which pacemaking is a function of the four small cells which trigger the five follower motor neurones *via* chemical and electrotonic synaptic excitation. The work reported here shows that there is strong feedback from motor neurones to small cells, by which endogenous burst-organizing potentials (driver potentials or DPs) and their hyperpolarizing afterpotentials contribute to regulation of bursting frequency. Isolated cardiac ganglia were placed in a two-chamber perfusion system which allowed independent treatment of small cells and motor neurones. When the motor neurones were silenced with tetrodotoxin (TTX), the small cells continued organizing bursts of activity which recurred at an average frequency 41% higher than bursting by normal ganglia in saline. The average burst duration was not altered. Driver potentials were evoked in TTX-treated motor neurones by electrical stimulation, by ionic alteration of the medium, or by treatment with the cardioexcitor peptide proctolin. DPs which occurred synchronously with small-cell bursts prolonged and intensified the bursts (more spikes per burst). When DPs were evoked in motor neurones during the interburst interval, they triggered small-cell bursts even at very short intervals after a spontaneous burst had occurred. All small-cell bursts which were associated with motor neurone DPs were followed by interburst intervals of longer than normal duration. The decrease in instantaneous burst frequency (increase in total burst cycle duration) caused by motor neurone DPs was similar in magnitude to the drop in burst frequency observed when the ganglion recovered normal activity after TTX washout.

Introduction

The cardiac ganglion of crustaceans, which initiates and coordinates the beat of the neurogenic heart, generates spontaneous rhythmic bursts of nerve impulses (for recent reviews, see Hartline, 1979; Tazaki & Cooke, 1983c; Benson & Cooke, 1984). In decapod crustaceans, the cardiac ganglion comprises two cell groups. Five large motor neurones send axonal processes out of the ganglion to innervate

Key words: cardiac ganglion, driver potential, feedback, pacemaking.

heart musculature. Four smaller 'pacemaker' neurones, also termed 'small cells', have their output processes confined to the ganglion (Hartline, 1967). It is now clear that the capacity of each of the nine intrinsic neurones to generate endogenous burst-organizing potentials (Tazaki & Cooke, 1979*b*, 1983*a,b,c*) is an important characteristic of the system. The burst-organizing potentials, termed driver potentials (DPs) by Tazaki and Cooke, are long-lasting depolarizations localized in a non-impulse conducting region of the neurone membrane.

The function of the cardiac ganglion has generally been viewed in terms of excitatory drive by the small cells on the motor neurones. The following brief summary of the physiology of the system is derived from the clear description of Tazaki & Cooke (1983*c*). After each burst the neurones of the system are in a relatively hyperpolarized state. A pacemaker potential, the mechanisms of which are incompletely understood, slowly depolarizes the cells during the interburst period. The small neurones depolarize more rapidly than the motor neurones during this period, and the voltage ultimately changes sufficiently to reach threshold for activation of driver potentials in the former cell group. The DPs in turn serve as a major source of depolarizing current which triggers impulse activity at distal spike-initiation zones. A combination of spike-mediated excitatory chemical synaptic input, electrotonic current flow between small and large cells and intrinsic pacemaker activity triggers nearly synchronous DPs in the five motor neurones. The DPs in these cells, which have been clearly localized to a region including the proximal axon (Tazaki & Cooke, 1983*b*), drive an impulse burst that, in intact systems, would cause heart contraction. Each burst is terminated by activation of a variety of potassium conductances which hyperpolarize the system to start the next cycle.

Analysis of the possibility of feedback influences of motor neurone activity on the pacemakers has been hindered by the extreme difficulty of recording from the latter cells. However, it has been known almost since the earliest studies on cardiac ganglia that currents passed into the motor neurones are able to influence the pacemaking characteristics of the system as a whole, presumably by acting *via* electrotonic connections (Watanabe, 1958; Watanabe & Bullock, 1960). Indirect evidence for an influence of motor neurones on pacemaker neurones has included the resetting of the burst phase by hyperpolarizing or depolarizing current pulses (Watanabe, 1958; Tazaki, 1972; Benson, 1980), the increased frequency of spike-mediated synaptic input to the motor neurones during motor neurone depolarization (Tazaki, 1971), the ability of intense electrically stimulated spike trains in motor neurone axons to trigger ganglionic bursts *via* the small cells (Mayeri, 1973*a*), and the observation that some neurohumours can alter the burst frequency of the system when only the responsive regions of motor neurones are exposed (Cooke & Hartline, 1975; Sullivan & Miller, 1984). These suggestions have been supported by more direct evidence from a very limited number of experiments where it has been possible to record simultaneously from a small cell and a motor neurone with microelectrodes (Tazaki & Cooke, 1979*a*). The possible functional role of feedback has been considered most fully by Benson (1980), who provided

evidence (mostly from studies involving phase resetting and neurohumoural alteration of DPs in crab ganglia) for the ability of large-cell activity to reinforce pacemaker-cell activity during a burst and for a role of the large cells in contributing to the rate of pacemaking during the interburst period.

The experiments cited in the preceding paragraph involve mainly the cardiac ganglia of crabs and the spiny lobster *Panulirus*. In the brachyurans, and probably in *Panulirus*, electrotonic connections between all cells appear to be strong (Tazaki & Cooke, 1979a; Tazaki, 1971). In contrast, electrotonic connections between cells in the cardiac ganglion of the lobster *Homarus*, at least between cell somata, have been thought to be relatively weak. The experiments reported here make use of a *Homarus* preparation in which the motor neurones are prevented from spiking, but are able to generate driver potentials, to demonstrate a significant feedback effect on small-cell activity. The results show that motor neurone DPs are capable of initiating small-cell bursts and of strengthening existing small-cell bursts. In addition, they demonstrate that the hyperpolarizing afterpotentials of motor neurone DPs make a substantial contribution to the pacemaker process during the interburst period, and thereby participate in frequency regulation of the system.

Materials and methods

Cardiac ganglia were isolated from the hearts of lobsters, and pinned through small bits of adhering muscle tissue to clear resin in a perfusion dish. A Vaseline barrier was placed across the trunk of the ganglion just anterior to the soma of the most posterior of the motor neurones (cell 5) (Fig. 1). The barrier served to separate the dish into two chambers which were perfused independently. The posterior chamber was perfused throughout each experiment with lobster saline (Welsh *et al.* 1968) containing $478 \text{ mmol l}^{-1} \text{ Na}^+$, $16 \text{ mmol l}^{-1} \text{ K}^+$, $26 \text{ mmol l}^{-1} \text{ Ca}^{2+}$, $8 \text{ mmol l}^{-1} \text{ Mg}^{2+}$, $546 \text{ mmol l}^{-1} \text{ Cl}^-$, $8 \text{ mmol l}^{-1} \text{ SO}_4^{2-}$ and 1 mmol l^{-1} glucose, buffered to pH 7.4 with 2 mmol l^{-1} Hepes, at 21°C . The anterior pool was perfused initially with normal saline. After an equilibration period during which normal activity was monitored, the perfusion fluid bathing the anterior chamber was switched to modified salines, as described in the Results section. When Ba^{2+} -substituted saline was used, SO_4^{2-} was eliminated from the perfusion fluid; exposure to Ba^{2+} was preceded by 0.5 h of perfusion with sulphate-free saline with otherwise normal ionic constitution.

Extracellular recordings of impulse activity in the ganglionic trunk were made with chlorided silver wire electrodes in the anterior and posterior chambers, across the electrical resistance provided by the Vaseline barrier. In such recordings, the activity of the small pacemaker neurones, in which impulses propagate anteriorly across the barrier, and of the large motor neurones in which impulses travel posteriorly in this region, could be distinguished on the basis of spike amplitude and the nature of the biphasic waveform of each spike. In each preparation an intracellular microelectrode recording was made from one of the four motor

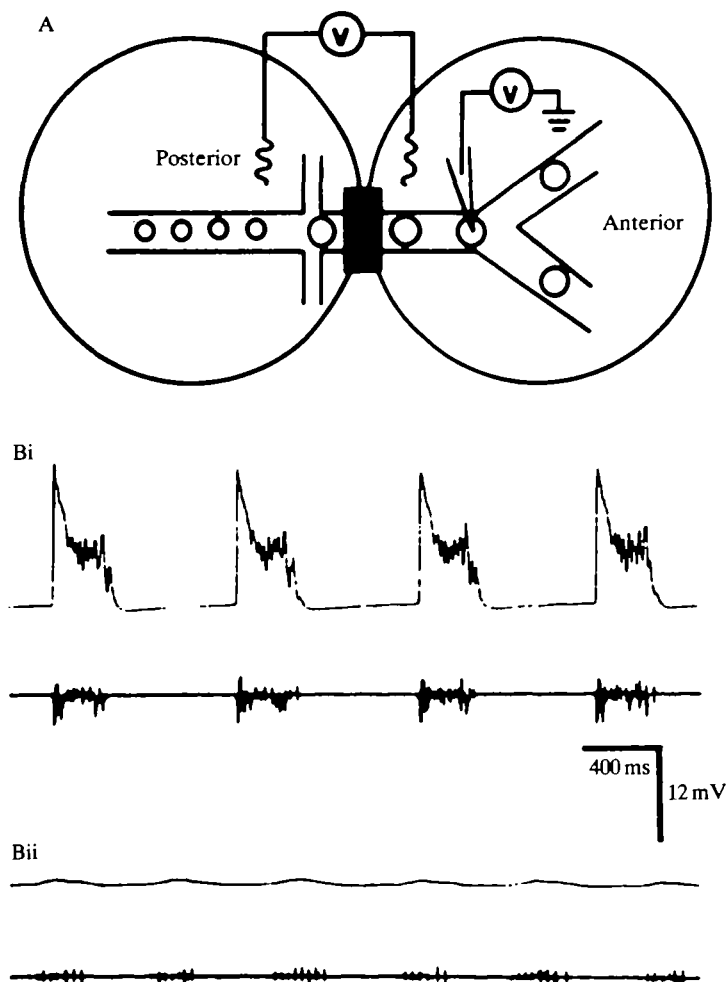


Fig. 1. (A) Lobster cardiac ganglion two-pool preparation. (Bi) Recording from cell 3 and trunk, with anterior and posterior ends in saline. In all figures, the top trace of each pair is an intracellular recording from the soma of one of the motor neurones in the anterior pool, and the bottom trace is the extracellular recording across the Vaseline barrier (dark bar). The early phase of each burst in such a record represents a strong contribution from the endogenous driver potential. (Bii) The same preparation after transfer of the anterior end to saline containing $1.5 \times 10^{-7} \text{ mol l}^{-1}$ tetrodotoxin (TTX). Note the increased burst frequency, weak extracellular bursts of shorter average duration, and low-amplitude residual input to cell 3. In all chart records, the vertical calibration refers to the intracellular trace.

neurone somata in the anterior chamber. Recordings were made with a Getting model 5 amplifier which allowed current-passing through the electrode. Ganglionic activity was monitored continuously on a Gould chart recorder. Burst durations and interburst intervals were measured on high-speed sections of the chart.

Results

Effects of tetrodotoxin in the anterior pool

Perfusion of the anterior pool with saline containing $1.5 \times 10^{-7} \text{ mol l}^{-1}$ TTX abolished impulse activity anterior to the barrier and indirectly eliminated the driver potential component and synaptic contributions normally recorded from the motor neurone somata. Bursting of the pacemaker neurones, with somata in the posterior chamber, persisted. Analysis of spike size and waveform confirmed that all persistent activity consisted of spikes within the posterior pool propagating anteriorly towards the barrier. Placement of the Vaseline barrier was critical to the maintenance of patterned small-cell activity. With the barrier just anterior to the soma of cell 5, the spike initiation zones of all five motor neurones would be expected to be in the anterior pool, whereas the trigger zones of three of the small cells should be in the posterior pool (Hartline, 1967). With the barrier placed slightly more posteriorly, so that only the trigger zones of the most posterior two small neurones would reliably be in the posterior saline pool, coordinated bursting was usually not observed. When TTX was added anteriorly to such a preparation, the ganglion either fell silent or small-cell impulses were propagated in a nonbursting pattern at low frequency.

In each of 36 preparations in which TTX was perfused anteriorly, the frequency of bursting was higher when the anterior end of the ganglion was silenced by TTX than when the whole ganglion was in saline (Fig. 1). The average burst frequency increased from 56 min^{-1} (range $32\text{--}92 \text{ min}^{-1}$) to 77 min^{-1} (range $40\text{--}120 \text{ min}^{-1}$). The average percentage increase, calculated from the change in each of the 36 preparations, was $41 \pm 4\%$ (mean \pm s.e.). As TTX was taking effect in the anterior chamber there was, in some preparations, an abrupt loss of the driver potential component recorded from the motor neurone soma. When this occurred, there was a corresponding abrupt increase in the frequency of bursting. More typically, preparations showed a gradual decrease in amplitude of the depolarization recorded in the motor neurone, and an upward creep in frequency during this period. After all motor neurone spike activity had been lost, there was a gradual further slight increase in frequency and weakening of the residual small-cell bursts, which stabilized after a few minutes.

In contrast to the uniform effect on burst frequency, the effect of silencing the anterior end of the ganglion on burst duration was variable. In 14 of the preparations burst duration increased, in two it was unchanged, and in the remaining 20 it decreased. The average burst duration decreased slightly from 274 ms (range 120–520 ms) to 267 ms (range 104–540 ms). The average percentage change in duration, $-1.5 \pm 4.1\%$, was not significant. Oscilloscope records of typical single bursts are shown in Fig. 2. There was no correlation between the initial burst duration in saline and the direction of change of duration during the subsequent exposure of the anterior end to TTX. As has been noted previously (Hartline, 1979), the normal burst in saline was initiated and terminated by small-cell discharge (Fig. 2), so that changes in overall extracellularly recorded durations reflected changes in time course of small-cell activity during the burst. With a

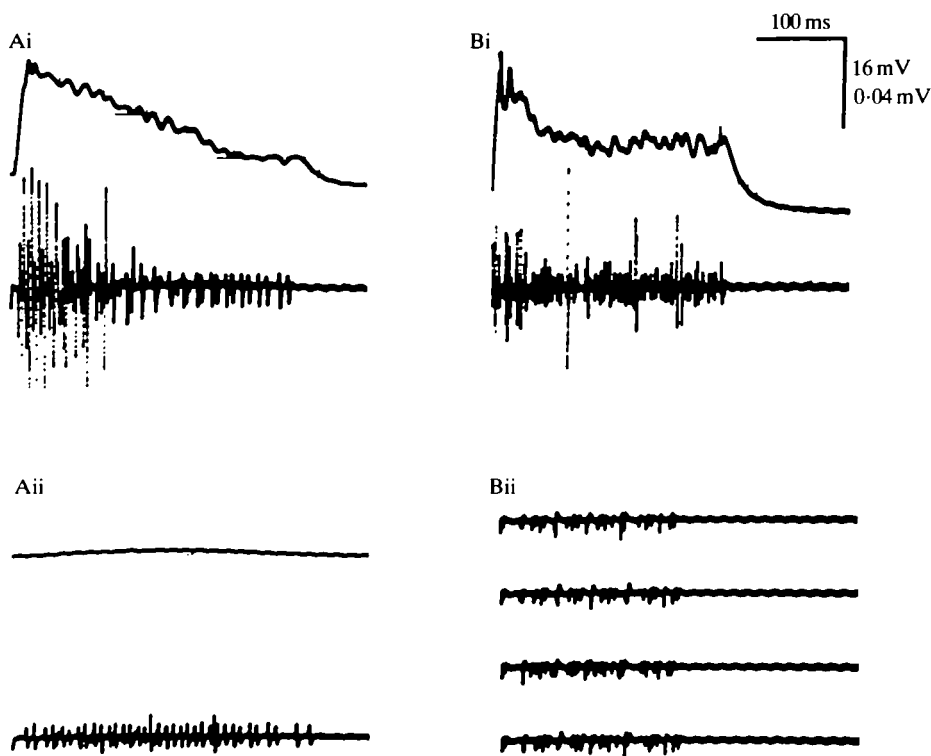


Fig. 2. Details of single bursts in two preparations, with the anterior end in normal saline (Ai and Bi), and in saline + TTX (Aii and Bii). Note that in saline each burst begins and ends with small-neurone (pacemaker cell) discharge, and in TTX only small-cell impulse activity persists. (Ai, Aii). Intracellular record from cell 4. The small-cell burst is longer in TTX than in saline. Low-amplitude input (<1 mV) persists to cell 4, appearing as the slight rise (lasting about 200 ms) and fall of the trace during the period corresponding to the burst. (Bi) Intracellular record from cell 1. Small-cell bursts were shorter than in saline. Four successive extracellularly recorded bursts are illustrated in Bii. There was no residual input to cell 1. Upper calibration refers to intracellular trace, lower to extracellular trace.

single site of extracellular recording it was not possible to discriminate with complete accuracy the activity of the different small neurones. However, in most preparations it appeared that all active small cells continued to fire in a coordinated fashion, throughout the period of discharge (e.g. Fig. 2B). It is possible, however, especially where residual activity was weak, that increases in duration of extracellular discharge reflected, in part, a loss of tight coordination between small cells.

In some preparations, the intracellular records from the TTX-treated motor neurones showed small depolarizations (less than 1 mV in amplitude) corresponding with each pacemaker-neurone burst (Figs 1Bii, 2Aii). This residual input was most often seen in the most posterior two of the four somata in the anterior

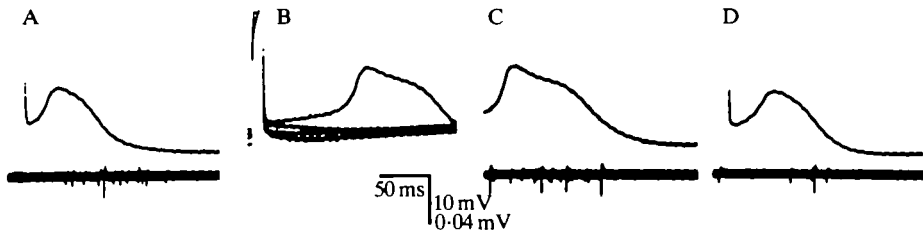


Fig. 3. Oscilloscope records of driver potentials in cell 1 (upper traces) and small-cell bursts. Anterior pool contained TTX in all records. A and D show driver potentials evoked by depolarizing current in normal saline. Note small-cell discharge triggered by the motor neurone driver potential. B shows a driver potential evoked by depolarizing current in choline-substituted saline (one of four stimuli was effective). The driver potential is longer and slightly larger than in saline. (C) A driver potential triggered by a small-cell burst in choline-substituted saline.

chamber (cells 3 and 4) and rarely from the most anterior two motor neurones (cells 1 and 2). Since the small-neurone axons projecting into the anterior chamber are silenced by TTX under these conditions, the low-amplitude residual input probably represents events occurring in the posterior chamber, recorded with attenuation through electrotonic connections.

Effects of driver potentials generated by motor neurones on pacemaker cell activity

With the anterior chamber in TTX, driver potentials could be evoked in motor neurones by passing brief depolarizing current pulses through the recording microelectrode, by altering the ionic composition of the medium, or by perfusing appropriate neurohumours through the anterior chamber. In most preparations it was not possible to evoke full driver potentials by passing current when the perfusion fluid was TTX-saline with normal ionic composition. In preparations where DPs were seen under these conditions they had high thresholds. Because of the rarity of observations of DPs in normal medium plus TTX, descriptions of the results of these experiments will be deferred until after consideration of preparations in which DPs were consistently produced.

In nine preparations the anterior chamber was switched from normal saline plus TTX to TTX-containing saline in which the sodium concentration was reduced to half its normal level by equimolar substitution with choline (see Tazaki & Cooke, 1979c; Berlind, 1982). Choline perfusion usually depolarized the baseline potential in the motor neurones by up to 3 mV. In all preparations it was possible to evoke driver potentials (average amplitude 23 mV in cells 1 or 2) by passing depolarizing current pulses of 20 or 50 ms duration. In those cells where DPs were seen in both normal- Na^+ saline and choline-substituted saline, the threshold current for evoking the DP was dramatically reduced in the latter medium, and the amplitude and duration of the DP slightly increased (Fig. 3). In about one-third of the choline-substitution experiments, DPs occurred in the motor neurones without



Fig. 4. Chart record from a preparation with the anterior end in TTX and reduced sodium (choline-substituted). Intracellular recording from cell 1. Chart speed was increased near calibration bars (horizontal calibration refers to high-speed portion of record). In this preparation every third to fourth small-cell burst triggered a driver potential in the motor neurone. When these occurred, the small-cell burst was prolonged and intensified (arrow). The average interval to the start of the next burst was significantly increased.

electrical stimulation, but were triggered intermittently by small-neurone bursts (Fig. 4).

When DPs occurred, whether they were triggered spontaneously or electrically evoked by stimulating during a small-cell burst, the impulse burst in the posterior half of the ganglion was usually increased in duration and intensity (more spikes per burst) (Fig. 4) and the interval to the start of the next burst was always increased (Fig. 5A). The increase in interburst interval did not, however, depend upon an increase in duration of the preceding burst; in some preparations of this type durations of the bursts during which a DP also occurred were *not* increased, but the succeeding interval was still prolonged (Fig. 5Aii).

Driver potentials could also be evoked by passing current into the motor neurone soma during the period between small-neurone bursts. These DPs in the anterior part of the ganglion were capable of initiating impulse bursts in the posterior half (Fig. 3), even at short intervals after a spontaneous burst had occurred. Analysis of the impulse waveform and size showed that the evoked impulses were conducting anteriorly towards the barrier and were therefore likely to be axonal impulses in the small neurones rather than spikes initiated in the distal motor neurone axons. Small-cell spiking was typically initiated shortly after the peak of the evoked motor neurone DP (e.g. Fig. 3A). Bursts evoked during the normal interburst period were usually shorter and weaker than spontaneously occurring bursts, but the succeeding interval was longer than normal.

When DPs were generated in the motor neurones in choline-substituted saline, the depolarizing phase was followed by a hyperpolarizing afterpotential lasting up to several hundred milliseconds. The motor neurone somata typically became about 2 mV more negative during the interburst period following a DP than during intervals following small-cell bursts with no DPs occurring in the motor neurones (Fig. 4).

Driver potentials could also be readily evoked by perfusing the anterior end of the ganglion with TTX-containing saline in which 50 % of the Ca^{2+} had been

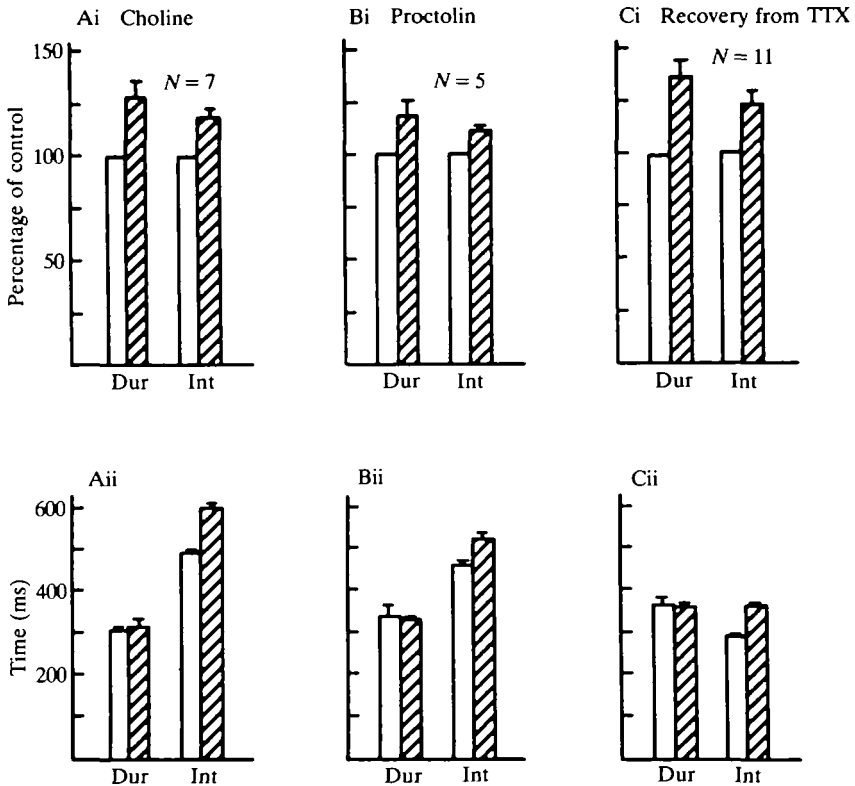


Fig. 5. Summary of small-cell burst durations (Dur) and interburst intervals (Int) showing the effects of motor neurone activity. Clear bars show control parameters when only small-cell activity occurs. Hatched bars show the influence of motor neurone driver potentials, evoked by anterior choline substitution (A) or anterior proctolin (B), and the influence of large neurone driver potentials plus action potential discharge during the period while the anterior end was recovering from TTX (C) (see text). The top series of histograms (Ai,Bi,Ci) shows the averages of all preparations (number indicated). The control periods (small-cell activity only) are normalized to 100, since burst durations and intervals varied considerably between preparations. Large-cell activity significantly increased the interburst interval in all preparations, and each treatment increased the average small-neurone burst duration. The lower histograms (Aii,Bii,Cii) show results of a selected single experiment of each type, in which the average burst duration was *not* increased by large-neurone activity. In each of these experiments, the average interval to the next small-neurone burst increased significantly, despite the constancy of the burst duration. Bars indicate standard error.

replaced with Ba^{2+} (Fig. 6). Barium substitution caused a baseline depolarization of 1–2 mV. Spontaneous DPs were not observed in any of three preparations which were exposed to Ba^{2+} for periods of up to 1 h. Depolarizing current pulses readily evoked DPs in the presence of Ba^{2+} that were 24–26 mV in amplitude (measured from the prestimulus baseline), about 250 ms in duration, and were followed by an afterhyperpolarization of about 2 mV. The effects of these DPs on

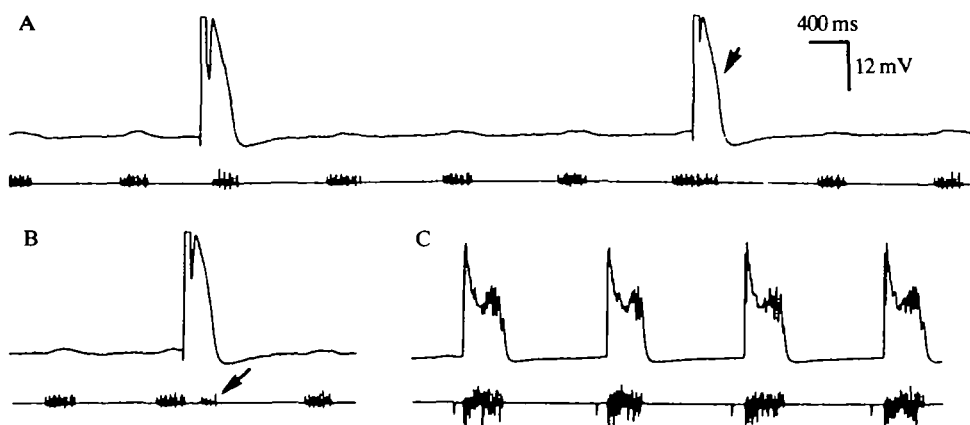


Fig. 6. Chart records of effects of driver potentials evoked by depolarizing current in Ba²⁺-substituted saline. Intracellular records from cell 3. A driver potential induced during a small-neurone burst (arrow in A) prolongs the burst. A driver potential evoked shortly after a spontaneous burst has occurred can trigger another (weak) burst after a very short interval (arrow in B). Average interburst intervals are increased whether the bursts associated with the driver potentials are shorter or longer than normal. C shows bursting in normal saline, in the same preparation after washout first of Ba²⁺ and then of TTX. The square leading edge of the driver potentials in A and B is a stimulus artefact.

small-cell activity were similar to those described above; that is, they triggered weak, short-duration small-cell bursts at very short intervals (Fig. 6), they prolonged small-cell bursts if they were elicited during existing activity, and they prolonged the interval before the next burst irrespective of the details of the pacemaker bursts with which they occurred. Effects of Ba²⁺ on DPs were completely reversible over the relatively short time course of exposure used in these experiments.

Motor neurone driver potentials were also observed in five of seven preparations following a pulse application of 10^{-6} mol l⁻¹ proctolin (50 μ l introduced into the anterior perfusion stream just before it entered the chamber). In these experiments TTX was present, the ionic composition of the medium was normal, and no electrical stimulation was applied. Proctolin at this dosage depolarized the baseline by an average of 5 mV. At the peak of the response motor neurone DPs usually occurred during every small-neurone burst (usually late in the burst). During the onset of proctolin action and during its washout DPs occurred intermittently in conjunction with some small-cell bursts (Fig. 7). The interburst periods were characterized by a steep pacemaker potential recorded from the motor neurone soma, with a clear inflection indicating the start of DPs (see also Sullivan & Miller, 1984). The amplitude of the DPs induced by proctolin averaged 11 mV (the amplitude of spontaneous DPs was measured from the inflection point at the end of the 'pacemaker' potential) and the membrane became about 2 mV more negative after a DP than after a burst when no DP occurred. In four of the

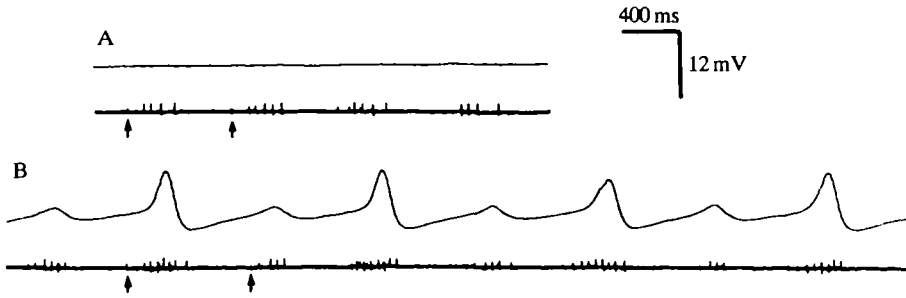


Fig. 7. Chart records showing the effects of a pulse of proctolin in the anterior pool. Intracellular recordings from cell 3. (A) Control activity before proctolin addition. (B) Activity recorded 3 min after introduction of proctolin. Note enhancement of the small-cell bursts which coincide with motor neurone DPs, and the delayed onset of the next small-cell burst after a motor neurone DP (arrows mark onset of extracellular activity for two bursts in each period). TTX is present in the anterior pool in both records.

five preparations, small-cell bursts associated with motor neurone driver potentials were increased in duration and intensity. Whether or not bursts were prolonged, the interburst interval was longer following a motor neurone DP (Fig. 5B).

The results of experiments in which it was possible to evoke DPs in saline of normal ionic composition are consistent with those of experiments with choline-substituted saline, Ba^{2+} -substituted saline or proctolin addition, although somewhat less conclusive. Five such preparations were obtained. Driver potential amplitudes recorded in cell 1 or 2 averaged 18 mV, with a 2 mV afterhyperpolarization. In all five preparations, the average interburst interval following an evoked DP was longer than the average interval between small-cell bursts in the absence of DPs. However, the lengthening of the interburst interval was statistically significant in only one of the experiments. (In all such preparations, the threshold for DP activation was very high, and the strong depolarization produced by a just subthreshold current pulse itself produced a small afterhyperpolarization and a slight increase in interval. The statistical comparison referred to above is therefore between interburst intervals following a DP induced by just suprathreshold current and intervals following a just subthreshold stimulus pulse.) In two of the five preparations a motor neurone DP evoked by electrical stimulation during the interburst period clearly triggered a small-cell burst (Fig. 3A,D).

Influence of motor neurone activity on pacemaker-neurone discharge during recovery from TTX

In all the experiments discussed above, there were no impulses conducted in motor neurone axons, and any influences of motor neurones on pacemaker-cell

activity would have to be mediated by communication not involving spikes. It was possible to observe the combined effects of motor neurone DPs and impulses during the washout of TTX. Normal burst activity was completely restored within 1 h of removal of TTX from the perfusion fluid. Initial stages of recovery were marked first by the abrupt appearance of motor neurone DPs triggered by a small percentage of the pacemaker-neurone bursts (Fig. 8Aii,B). As washout proceeded there was a gradual increase in the percentage of small-cell bursts which triggered motor neurone activity, until the latter finally occurred with every burst. Whenever motor neurone DPs occurred during this recovery, they were accompanied by a brisk action potential discharge in motor neurone axons.

When motor neurone activity occurred, the total burst duration (now including impulses in both cell types) was usually increased. The motor neurone intracellular record showed an afterpotential making the cell soma several millivolts more negative than during bursts without motor neurone activity (Fig. 8), and the interval to the initiation of the next burst was significantly prolonged (Fig. 5C). In some preparations (Figs 5Cii, 8B) intervals were prolonged without a change in burst duration.

The results reported above show that motor neurone DPs tend to prolong existing bursts in small cells and to increase the interval to the next burst, thereby increasing the duration of the total cycle (or decreasing the instantaneous frequency). It is of interest to determine whether the addition of motor neurone impulses to the DP has any further effect on overall cycle period and frequency. Although it was not possible to resolve this definitively because of long-term changes in small-cell burst characteristics between the trial in TTX and the recovery period in most experiments, the results of a few individual preparations are useful. In each of the two experiments illustrated in Table 1, the small-cell burst characteristics were similar during the two experimental periods. The percentage decrease in instantaneous frequency caused by DPs (induced by proctolin in preparation I and by electrical stimulation in choline-saline in preparation II) was similar in magnitude to the change caused by DPs plus impulses during recovery. These results suggest that most if not all of the influence of motor neurone activity on pacemaking characteristics of the ganglion is attributable to the locally generated driver potentials and their aftereffects, rather than to impulse-mediated events. It is also noteworthy that the addition of DPs alone was sufficient in most cases to slow the burst frequency of the small cells to a level close to that exhibited by the intact ganglion bursting in normal saline.

In some preparations the events of the recovery period suggested long-lasting aftereffects of motor neurone activity which could influence the small-cell bursts through several cycles. The traces shown in Fig. 8C show a period during recovery when the motor neurones were triggered during only every fourth burst. The three small-cell bursts between motor neurone activations grew progressively stronger. Burst durations and numbers of impulses per burst increased sequentially, as did the amplitude of the synaptic depolarization caused in the motor neurones by small-cell activity.

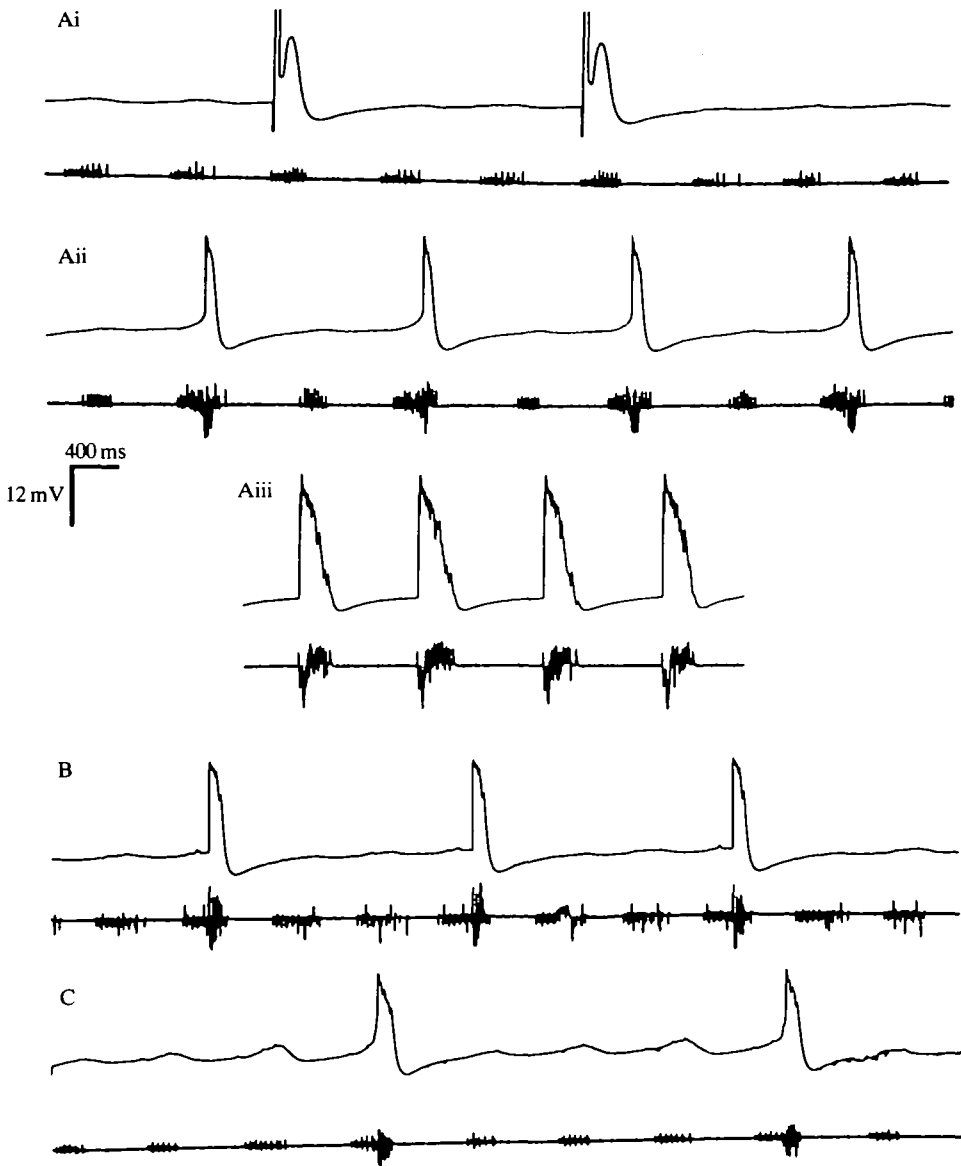


Fig. 8. (A) A comparison of the effects of driver potentials induced by current in choline-substituted saline (Ai), of periodic motor neurone activation occurring during washout of TTX from the anterior pool (Aii), and of normal bursting in saline (Aiii). Intracellular records from cell 3. Note that driver potentials (record i) are considerably smaller in amplitude and shorter in duration in this preparation than the peak depolarizing phase of the discharge in normal medium (record iii), as are the intermittent bursts occurring during recovery (record ii). (B) Intracellular record from cell 4. In this preparation, bursts were of equal duration whether motor neurone discharge was triggered or not, but interburst intervals were significantly prolonged following activation of the large cells. (C) Intracellular recording from cell 4 (different preparation) during recovery from TTX, showing gradual enhancement of small-cell bursts, and their subthreshold input to the motor neurones, in the period between large-cell bursts.

Table 1. *Influence of motor neurone DPs alone or of motor neurone DPs plus impulse activity on burst characteristics*

	Motor neurones in TTX		Motor neurones recovering from TTX	
	Small-cell activity only	Small-cell activity plus motor neurone DPs	Small-cell activity only	Small-cell activity plus motor neurone DPs and impulses
Preparation I				
DPs induced by:		Proctolin		
total cycle length	701 ms	842 ms	739 ms	885 ms
corresponding burst frequency	86 min ⁻¹	71 min ⁻¹	81 min ⁻¹	68 min ⁻¹
% change in frequency due to motor neurone activity		-17 %		-16 %
final frequency after full recovery				72 min ⁻¹
Preparation II				
DPs induced by:		Choline		
total cycle length	774 ms	946 ms	744 ms	985 ms
corresponding burst frequency	78 min ⁻¹	63 min ⁻¹	81 min ⁻¹	61 min ⁻¹
% change in frequency due to motor neurone activity		-19 %		-25 %
final frequency after full recovery				56 min ⁻¹

Discussion

Two major conclusions derive from the results described above: (1) The motor neurones of the *H. americanus* cardiac ganglion are capable of exercising considerable feedback influence on pacemaker-cell activity on a cycle-by-cycle basis, by reinforcing existing activity during the burst and by prolonging the pacemaker period between bursts; (2) the feedback effects can occur in the absence of any impulse activity by the motor neurones and appear to be mediated largely, if not exclusively, by electrotonic current flow or nonspiking transmission from motor neurone driver potentials and their afterpotentials. These conclusions support those drawn by Benson (1980) from studies of a crab cardiac ganglion and demonstrate similar effects in a ganglion where coupling between neurones had been thought to be considerably weaker. In addition, the current experiments involve a situation where the motor neurones are prevented from spiking by TTX,

and the bursts that are being modified involve exclusively small-cell activity. These results therefore allow an unequivocal attribution of observed effects on small cells to the motor neurone DP itself. In previous studies, in contrast, changes in burst parameters involved compound bursts, with both large- and small-cell impulse activity.

The contribution of large-cell activity to the duration of the interburst period and to burst frequency was observed consistently in diverse experimental situations. Silencing the large cells with TTX caused a significant increase in burst frequency with very little change in average burst duration. Mayeri (1973a) observed an increase in small-cell burst frequency in *Homarus* when the small-cell group was isolated from the motor neurones by transection of the ganglion between cells 5 and 6. In individual experiments in the current series, the interval between small-cell bursts and its inverse, the instantaneous burst frequency, could be restored to levels close to that of full bursts in normal medium simply by the coincident occurrence of motor neurone DPs (Table 1). These results suggest that the pacemaker process which determines the interval between bursts in normal ganglia includes a significant contribution from events occurring in the motor neurones. The exact nature of the pacemaker potential, which slowly depolarizes the system between bursts, is not known. The pacemaker process may include depolarization by nonspecific leakage, and involvement of an electrogenic pump mechanism (Livengood & Kusano, 1972). However, consistent observations of a declining conductance during the interburst period (Connor, 1969; Livengood & Kusano, 1972) strongly implicate a contribution from the turning off of a Ca^{2+} -activated K^{+} conductance which is known to be activated following Ca^{2+} entry during driver potentials (Tazaki & Cooke, 1979c, 1986). The results here support the notion that pacemaking in the system as a whole is a widely distributed process that is not restricted to the so-called pacemaker neurones, but that under normal circumstances has inputs from the motor neurones as well (see also the discussion of Sullivan & Miller, 1984). The small cells in this model are normally the effective pacemakers; each burst starts with small-cell activity because, under the influence of the network, they depolarize more rapidly than the motor neurones and/or have a much lower threshold for activation of their DPs and spikes.

The experimental techniques used here do not allow an unequivocal determination of whether the feedback pathway from motor neurones to small cells involves electrotonic connections or graded, nonspiking chemical transmission. In the related crustacean stomatogastric ganglion, where intracellular recording from both pre- and postsynaptic elements is feasible and where chemical interactions are inhibitory, clear evidence has been presented that voltage changes similar to DPs can influence other neurones through graded transmission (Graubard *et al.* 1980). The most detailed electron-microscopic study of cardiac ganglia, made in the crab *Portunus sanguinolentus*, suggests that the morphological basis for both communication modes may be present (Mirolli *et al.* 1987).

With ganglia in normal saline, d.c. hyperpolarizing or depolarizing currents passed into somata are more effective in altering burst frequency if passed into the

more posterior motor neurones than into those that are more anterior (A. Berlind, unpublished observations). This gradient of effectiveness is consistent with a smaller electronic distance from the more posterior somata to a critical region for pacemaking. In contrast, DPs evoked in any of the four motor neurones used in the experiments reported here are equally effective in altering small-cell activity. Simultaneous intracellular recordings from two cells were not made here, but have been made in other studies, with a ganglion in TTX in a single perfusion pool. In this situation, spontaneous DPs or DPs activated by neurohumours always occur synchronously in any pair of motor neurones, and DPs evoked by brief current pulses in any cell invariably trigger DPs in any of the other four (A. Berlind, unpublished observations). DP-generating zones therefore appear to be very tightly coupled.

The depolarizing phase of motor neurone DPs, without associated impulses, is able to act through the network to reinforce existing small-cell bursts or to trigger such bursts prematurely in the interburst period. It cannot be determined from these experiments whether the motor neurone DP is triggering small-cell impulses directly by causing depolarization of the spike-initiating zones of the small cells, or indirectly, by enhancing or triggering small-cell DPs. If the latter is the case, the observations that small-cell bursts can be triggered at very short intervals after a spontaneous burst has occurred is of interest with regard to the possible differences in mechanisms of the DPs in the two cell types. As noted previously, the DPs in the small cells have not been analysed directly, but indirect evidence suggests that they have a distinctly different time course (less rapid rise and fall) in the small cells (Tazaki & Cooke, 1979a), different ionic dependencies (Berlind, 1985), and different modulation by neurohumours (Benson, 1980; Miller *et al.* 1984; Sullivan & Miller, 1984). Tazaki & Cooke (1979b, 1983b) have shown refractoriness for motor neurone DPs in both the crab *Portunus sanguinolentus* and the lobster *Homarus americanus*, in that depolarizing stimulus pulses cannot evoke DPs in these cells shortly after one has occurred, and that even after longer intervals DPs have higher thresholds or reduced amplitudes. If the short-interval bursts (e.g. Fig. 6) seen here do represent the interposition of a small-cell DP between motor neurone DP and small-cell burst it would indicate that the pacemaker-cell DPs show little or no refractoriness.

My results differ significantly from those of Benson in at least one particular: the prolongation of the interval between small-cell bursts caused by motor neurone DPs did not require a prolongation of the preceding bursts. Although motor neurone DPs typically prolonged small-cell bursts, similar interburst effects were observed in individual preparations where the correlated burst was not prolonged (Fig. 5) or where the bursts were much shorter in duration than normal (Fig. 6). The effect of DPs in prolonging the interburst intervals seems therefore to be more closely correlated with the occurrence of the hyperpolarizing afterpotential than with the burst duration *per se*. As Benson has pointed out, under normal bursting conditions the duration of the afterhyperpolarization is likely to be correlated with burst duration if enhanced Ca^{2+} inflow occurs during the prolonged plateau and

the Ca^{2+} -activated K^{+} conductance is thereby enhanced. However, there are clearly experimental situations, particularly involving neurohumour treatment, where the normally strong inverse correlation between burst duration and burst frequency (Mayeri, 1973b; Benson, 1980) does not hold, but rather where both burst frequency and burst duration increase together (Cooke & Hartline, 1975; Lemos & Berlind, 1981; Miller *et al.* 1984). Any overall model of burst formation and pacemaking must therefore include an explanation of these observations. It is worth noting that the burst durations monitored by Benson in *Portunus* were apparently plateau durations from the intracellular motor neurone records. In *Homarus*, the similar record is of compound origin, with the early peak phase of depolarization dominated by the motor neurone DP contribution and the later plateau representing primarily continued synaptic drive (EPSPs) from the small cells (Hartline & Cooke, 1969; Tazaki & Cooke, 1983c; Berlind, 1985). It is not safe to assume, therefore, that the duration of the motor neurone intracellular plateau is invariably correlated with the amount of calcium entry.

The addition of motor neurone action potentials to the DP seems to have little or no effect on small-cell bursting. This finding is consistent with the conclusions with regard to feedback effects in *Portunus* (Benson, 1980) and *Panulirus* (Otani & Bullock, 1959; Tameyasu, 1976), and with other studies on *Homarus* showing that evoked spike trains in motor neurone axons have little effect on the system as a whole (Mayeri, 1973a; Cooke & Hartline, 1975). In this system, as in others, it is clear that rapid potential changes are much more severely attenuated than slow potentials (Hagiwara *et al.* 1959; Watanabe & Bullock, 1960; Tazaki & Cooke, 1979a).

I thank Drs I. M. Cooke and D. Bodznick for helpful comments on an earlier version of this paper. This research was supported by a grant from the Whitehall Foundation.

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