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THE CARDIOREGULATORY SYSTEM OF CRAYFISH: THE ROLE OF CIRCUMOESOPHAGEAL INTERNEURONES

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SUMMARY

1. Interneurones located in the circumoesophageal commissures were found to control the activity of the cardioinhibitor and cardioaccelerator regularity neurones.

2. These interneurones (cardiac command fibres) fell into three classes: (1) strong inhibitors, which caused cardiac arrest, (2) weak inhibitors, which caused bradycardia, and (3) accelerators, which caused tachycardia.

3. When the positions of interneurones were plotted collectively, they formed distinct clusters, suggesting that each cluster was represented by one command unit in an individual preparation. Twenty strong inhibitor units and 16 accelerator units were found. Weak inhibitors did not form clusters.

4. Stimulus threshold characteristics were as low as 3·0 V and 3 Hz for the strong inhibitor units (mean range 4·7–7·1 V and 14–30 Hz). Higher values were found for weak inhibitors and accelerators.

5. The strong inhibitor command drives always showed a positive bias toward the contralateral cardioinhibitor neurone, relative to the ipsilateral cardioinhibitor.

6. Plots of command neurone stimulating frequency versus evoked cardioinhibitor activity displayed steep positive slopes for strong inhibitor command units and shallow positive slopes for weak inhibitor units.

7. Reciprocity between the cardioinhibitor and cardioaccelerator neurones occurred during both inhibitory and acceleratory command drives. This is not likely to be a property inherent in the command units themselves because reciprocity was earlier observed during chemical and tactile reflex inhibition of the heart. (Field & Larimer, 1974*a*).

INTRODUCTION

Wiersma & Novitski (1942) described a single interneurone, in the crayfish circumoesophageal connective, which was capable of driving cardiac arrest when stimulated. Although circumoesophageal command fibres which drive overt behavioural patterns were subsequently described by Atwood and Wiersma (1967) and by Bowerman & Larimer (1974*a*, *b*), the interesting possibility of cardiac 'command fibres' has remained largely neglected since Wiersma and Novitski's original description of the inhibitory neurone. Recently, Wilkens, Wilkens, & McMahon (personal communication) searched for circumoesophageal interneurones which controlled cardiac and scaphognathite rhythms in *Cancer*. Sixty-eight% of the stimulated bundles which

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affected the scaphognathites also affected the heart, although the effect of frequency often differed for the two systems.

The present investigation extends the analysis of circumoesophageal neurones which exert central nervous control upon the cardioregulatory neurones. Our previous paper described the anatomy, identification and spike activity of the cardioregulator neurones during reflex control of the heart in crayfish (Field & Larimer, 1974*a*). The present analysis deals with (*a*) the number and position of 'command' neurones in the circumoesophageal commissures which affect the heart rate, (*b*) the action of these interneurones upon the cardioinhibitor and cardioaccelerator neurones, and (*c*) possible circuitry between the circumoesophageal and the cardioregulator neurones.

METHODS

Adult crayfish (*Procambarus clarkii*) were dissected and mounted in a cooled, oxygenated bath of van Harreveld's saline as described in the previous paper (Field & Larimer, 1974*a*), except that the pericardium was not exposed. Recordings were made from the cardioregulator nerves with suction electrodes and from the heart with silver bipolar electrodes inserted through holes in the carapace (Larimer, 1962). The right circumoesophageal commissure was used exclusively. After crushing both commissures near the brain to eliminate descending activity, the right commissure was desheathed and the medial and lateral giant axons were removed. Fine bundles of axons were stripped from the connective with sharpened watchmakers' forceps and wrapped around a pair of 50 μm platinum stimulating electrodes. These leads were insulated except for the terminal 1 mm and immersed in the bath near the connective. To allow comparison of voltage thresholds between bundles within one animal, and between animals, the same pair of stimulating electrodes was used throughout. Thus, constant field conditions were ensured. Stimulus intensities ranged from 1–10 V and 1–150 Hz. All accelerator fibres were located in preparations in which the inhibitor nerves (SN II) were sectioned to prevent interference from tonic or spontaneous inhibition.

Electrical impulses were amplified and displayed by conventional means. The ECG signal was also fed into a cardiotachometer (Harvard Apparatus) and displayed as a d.c. analog of heart rate on one channel of an oscillographic pen recorder. All electrical data were stored on magnetic tape (Ampex FR-1300A recorder). Subsequent digital analysis of spike rates was carried out with Ortec Single Channel Analyzers (Model 730) and a Model 441 Ratemeter, and displayed on the pen recorder.

RESULTS

The initial step of the analysis was to determine the number, variety and location of units in the circumoesophageal commissure which exerted an effect on the heart rate when stimulated. Fig. 1 illustrates three classes of interneurones which modulated cardiac activity. The first class comprised 'strong' inhibitors (Fig. 1A) which produced cardiac arrest, even when stimulated at threshold frequencies. Usually the inhibitory effect outlasted the stimulus duration, which would be expected in view of the lengthy burst-discharge, characteristic of cardioinhibitors (Field & Larimer, 1974*a*). With prolonged stimulation, as in Fig. 1A, the heart often escaped the inhibi-

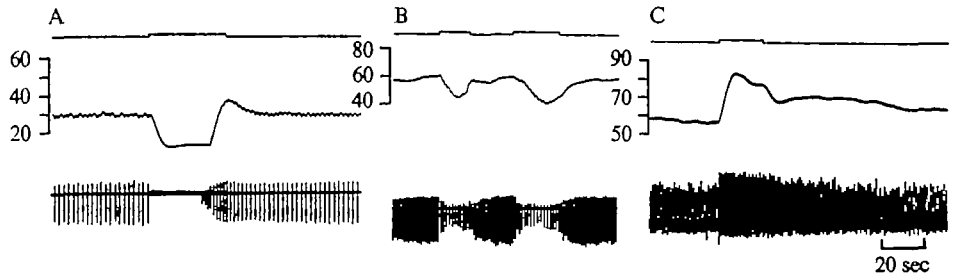

Fig. 1. The effects of stimulating inhibitor and accelerator circumoesophageal command interneurones on the heart rate. (A) Stimulation of a 'strong' inhibitor at 5 V, 20 Hz. Note cardiac arrest and post-inhibitory rebound in heart rate. (B) Stimulation of a 'weak' inhibitor with two stimulus trains (8 V, 40 Hz). The response is characterized by incomplete inhibition and lack of rebound. (C) Stimulation of an accelerator at 8·6 V, 60 Hz (the optimum frequency for this unit). Top trace, stimulus duration; middle trace, cardiotachometer output in heart beats/min; bottom trace, electrocardiogram.

tory drive, a phenomenon noted earlier by Wiersma & Novitski (1942), Maynard (1953) and Florey (1960) during stimulation of the cardioinhibitor nerves. The last author ascribed the return of cardiac activity to post-synaptic adaptation in the cardiac ganglion cells. Post inhibitory rebound, also noted by the above authors, usually occurred following cardiac arrest caused by strong inhibitors (Fig. 1 A). Evidence from our previous report (Field & Larimer, 1974*a*) indicated that the 'rebound' effect may be partially due to increased cardioaccelerator activity following inhibition.

The second class of interneurones was represented by 'weak' inhibitors. These units produced only bradycardia, even at maximal stimulation frequencies, and their inhibitory effect was never observed to outlast the stimulus duration. As shown in Fig. 1 B, the heart often adapted to the inhibition after 5–10 sec and returned to its normal rate of activity during stimulation. Post-inhibitory rebound effects were seldom seen following weak inhibitor stimulation. The effectiveness of both strong and weak inhibitor interneurones did not appear to be correlated with heart rate.

The third class consisted of accelerator interneurones, the effect of which is shown in Fig. 1 C. We did not observe any distinct categories of accelerators, although their effectiveness varied between units and between preparations. The maximum increase in cardiac rate evoked by accelerator interneurone stimulation was 45% (from 60 to 87 beats/min). The heart rate either reached an initial peak followed by a reduced frequency plateau (cf. Fig. 1 C) or climbed with a diminishing rate toward a maximum frequency during stimulation. A long, steady decrease in heart rate (Fig. 1 C) usually characterized the return to base-line frequency. The prolonged return was also noticed by Wiersma & Novitski (1942), Maynard (1953) and Florey (1960), while directly stimulating the cardioaccelerator nerves.

In general, changes in the ECG amplitude were similar to those described for heart rate, except that the overshoot and rebound effects were not observed (cf. Fig. 1 A, B, C).

For every preparation, the position of each effective axon bundle was plotted on a cross-sectional map of the right connective similar to that shown in the inset of Fig. 2. The maps were divided into regions according to the numbering system (inset, Fig. 2) designated by Wiersma (1958). The loci from all preparations were

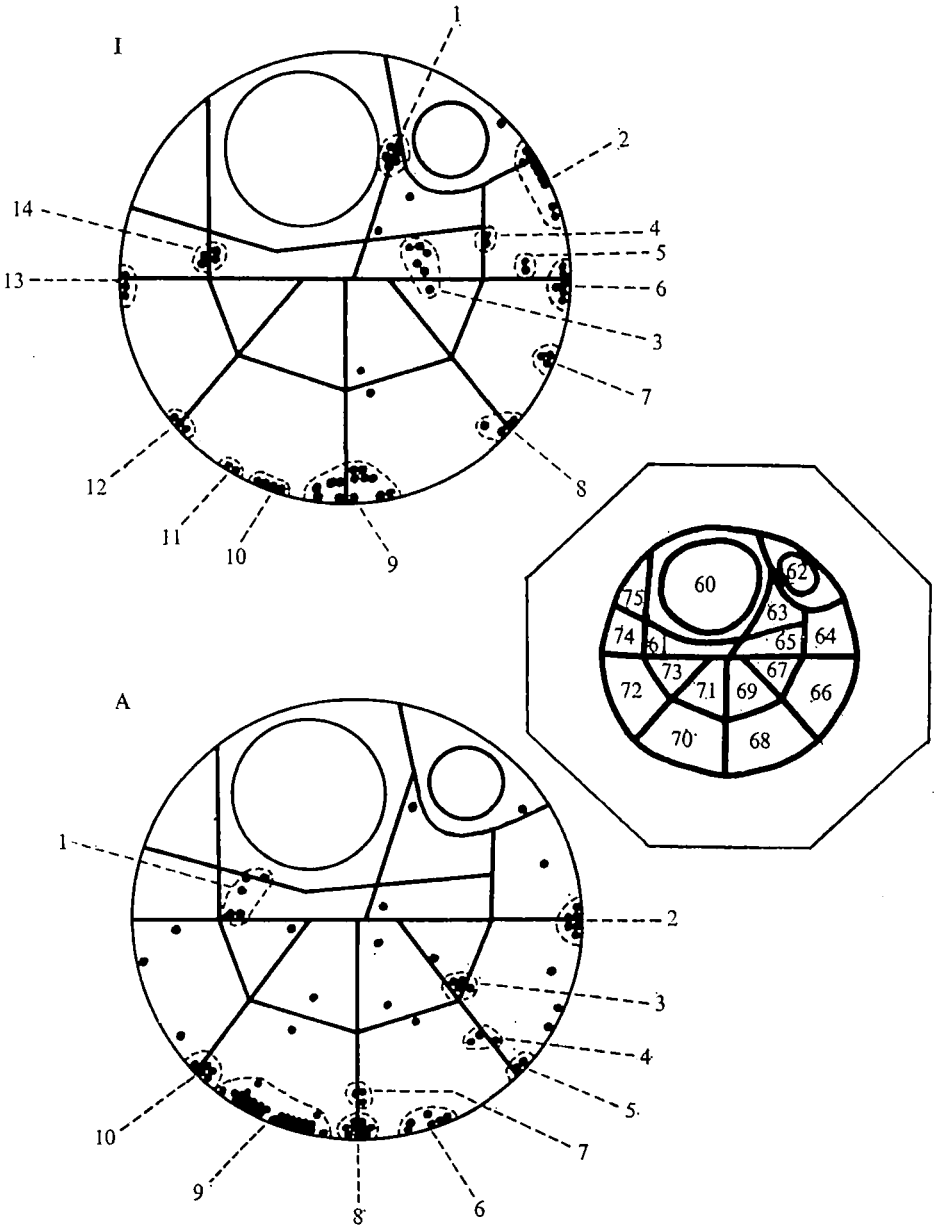

Fig. 2. The positions of cardiac command units mapped on a cross-section of the right circumoesophageal commissure. The inset shows the numbered area designated by Wiersma (1958) and referred to in this study. The upper map (I) shows the position of all strong inhibitors found (black dots) while the lower map (A) indicates the positions of all accelerator units (black dots) that were encountered. Since the positions fell into clusters on both maps it is likely that each cluster represented one (or in some cases, several) command units encountered repeatedly. Therefore, each cluster was outlined with a dotted line and assigned a command fibre locus number. Note the general restriction of loci to the periphery of the connective as well as an overlap of inhibitor and accelerator loci in some areas. Stimulation threshold data for each unit are presented in Tables 1–3.

Table 1. *Positions and characterization of strong inhibitory units found in the right circumoesophageal commissure*

c.f. locus	Area	No. times found	Threshold voltage (V)	Threshold frequency (Hz)	Comments
1	60–63	5	4·2–6·0	4–30	1 unit
2	64	9	3·4–5·6 4·6–6·8	3–5 20	2 units*
3	65	6	6·8–7·5 4·0–8·6	20–30 40	2 units*
4	64–65	2	4·0–8·0		1 unit
5	64	2	4·0–9·0	4	1 unit
6	64–66	5	3·0–7·2	10	1 unit, very low threshhold
7	66	3	4·0–7·3	10–50	1 unit
8	66–68	3	5·0–6·8	10–40	1 unit
9	68–70		3·5–10·0	5–50	At least 5 units*
10	70	4	4·8–6·8	5–30	1 unit
11	70	2	8·0–10·0	30	1 unit
12	70–72	3	3·8–5·4	10–15	1 unit
13	72–74	3	3·9–5·4	15–20	1 unit
14	61–74	4	3·6–6·0	20	1 unit

* Determined on the basis of the maximum number of units found at this locus per preparation.

compiled on the two maps shown in Fig. 2 for strong inhibitory command fibres (I) and acceleratory command fibres (A). Weak inhibitor fibres were not shown for reasons discussed below. The dashed lines enclose areas where a unit was found two or more times. Since the loci from different preparations tend to form distinct clusters, it is likely that each cluster represents one command fibre; it is therefore assigned a command fibre (c.f.) locus number. Exceptions to this conclusion are those clusters which contain two or more loci from the same animal. For example, some individual preparations yielded two units each in c.f. loci 2 and 3 on the inhibitor map, while c.f. locus 9 contained a maximum of 5 units from one preparation (see Table 1, strong inhibitory units). Similarly, c.f. locus 9 on the accelerator map contains at least 4 units (Table 3). This contrasts sharply with more easily identified units, such as c.f. locus 1 on the inhibitor map, which had one clearly recognizable strong inhibitor located between the two giant fibres. A total of 14 strong inhibitor and 10 accelerator c.f. loci were plotted. These regions contained approximately 20 strong inhibitor units and 16 accelerator units. Tables 1, 2, and 3 summarize the position and stimulation characteristics of the fibres.

The weak inhibitor units were much more scattered in location and did not fall into clusters. Some positions coincided with the loci of strong inhibitor units and hence it is not known whether these might have actually been strong inhibitors but with reduced synaptic efficacy. In general, however, weak inhibitors had higher thresholds which suggests that they were a separate class of fibre (see Table 2).

Two generalizations about the command fibre maps deserve mention. First, most

Table 2. *Position and characterization of weak inhibitor units found in the right circumoesophageal commissure*

c.f. locus	Area	No. times found	Threshold voltage (V)	Threshold frequency (Hz)	Comments
1	62–64	1	5·0	50	1 unit
2	64–65	1	9·0	40	1 unit
3	66	1	7·0	(40)*	1 unit
4	68	2	5–10	(40)*	1 unit
5	68–69	1	5	(40)*	1 unit
6	68–70	10	3·0–8·4	10–40	At least 3 units†
7	70	2	4·2–5·6	10–30	1 unit
8	70	2	5·0–7·2	40	1 unit
9	70–72	2	4·0–9·0	(40)*	1 unit
10	72	1	8·0	(40)*	1 unit
11	72	2	8·0–9·0	40	1 unit
12	74	2	7·8–9·0	15–40	1 unit
13	61	1	10·0	40	1 unit
14	60–75	1	8·0	(40)*	1 unit

* Signifies that these units were only stimulated at a frequency of 40 Hz.
† Determined by the maximum number of units found at this locus per preparation.

of the loci tend to occur on or near the periphery of the commissure. Although the significance of this is not immediately apparent, a functional distribution pattern may emerge when more command fibre positions are elucidated in the circumoesophageal commissures. Secondly, there is an apparent overlap in many of the inhibitor and accelerator loci, e.g. area 70 (see inset, Fig. 2). This was indirectly confirmed when teased axon bundles were occasionally found which evoked acceleration followed by inhibition. The component interneurones were sometimes demonstrated by additional subdivision of the bundle.

Tables 1, 2 and 3 present stimulus threshold characteristics of the strong inhibitor, weak inhibitor and accelerator command fibres. Although these values were subject to some variation due to differences in size of the teased bundles, other sources of variation were minimized by using the same pair of stimulating electrodes, the same electrode geometry and the same stimulus pulse duration throughout the analysis. A number of strong inhibitors had threshold voltages as low as 3·0 V, which suggests that these were relatively large axons. Furthermore, this class of interneurones was effective at extremely low threshold frequencies, often less than 10 Hz and, in a few cases, 3 Hz (Table 1). The mean ranges of activation values (Σ_{min}/N to Σ_{max}/N) were 4·7–7·1 V and 14–30 Hz. By contrast, the weak inhibitors usually required higher stimulus levels (Table 2). The mean range of threshold voltages was 6·4–8·3 V while the mean range of threshold frequencies was 31–37 Hz. The accelerator units also had somewhat higher threshold values than the strong inhibitors (Table 3). The minimum voltage level found was 3·6 V (mean range 5·4–8·1 V). In a few cases acceleration could be driven at 5 Hz, but the mean range of values was substantially higher (19–48 Hz)

In some instances stimulation of fine bundles only produced cardiac effects, but occasionally the stimulated bundle also mediated overt behavioural scores which closely followed the cardiac effect in terms of threshold voltage and frequency response.

Table 3. *Positions and characterization of accelerator units found in the right circumoesophageal commissure*

c.f. locus	Area	No. times found	Threshold voltage (V)	Threshold frequency (Hz)	Comments
1	61	5	6·0–8·0	20–40	2 units*
			6·0–9·0	40	
2	64–66	6	4·0–8·5	5–30	1 unit
3	66–67	4	4·0–9·0	20–60	1 unit
4	66–68	3	7·0–7·5	40–60	1 unit
5	66–68	2	5·5–7·0	5–20	1 unit
6	68	6	5·0–5·5	10–40	2 units*
			6·0–9·0	20–60	
7	68–70	3	6·8–9·0	20–80	1 unit
8	68–70	9	3·7–8·0	10–80	1 unit
9	70	29	5·0–7·0	20–60	At least four units*
			3·6–8·0	10–40	
			6·0–8·2	10–60	
			4·0–9·0	5–20	
10	70–72	5	7·8–9·0	60–70	2 units*
			5·2–8·5	5–10	

* Determined by the maximum number of units found at this locus per preparation.

Table 4. *Frequency responses of simultaneous cardiac and behavioural effects evoked by stimulation of an accelerator command unit (c.f. locus 9, area 70 in Fig. 2B)*

The threshold for both effects was 9 V and 10 Hz. The cardiac effect is expressed as change in beats per minute from pre-stimulus level to peak acceleration level.

Frequency (Hz)	Δ ECG (bpm)	Behaviour
5	None	None
10	+1·3	Slight abdominal flexion, slow leg flexion
40	+7·2	Faster abdominal flexion, faster leg flexion and elevation, cheliped flexion
60	+20·6	Full abdominal flexion, full leg flexion and elevation, cheliped flexion
80	+3·9	Full abdominal flexion, full leg flexion and elevation, cheliped flexion

An example of corresponding behavioural and cardiac responses is given in Table 4. The accelerator effect and concomitant leg and abdominal flexion had a threshold of 9 V and 10 Hz. As the stimulating frequency increased, greater acceleration occurred together with faster flexion movements. In addition, cheliped flexion and leg elevation (coxal) began to appear. Maximum cardiac and behavioural effects occurred at 60 Hz, above which acceleration diminished while the behaviour remained the same. The entire behaviour score is similar to the command-driven 'generalized flexion' behaviour, described by Bowerman & Larimer (1974*a*). In still other instances overt behaviours were evoked together with cardiac changes, but the behaviour did not have the same frequency response as the cardiac effect. In these cases the motor score would either drop out, become fractionated or a new behaviour would appear with changes in stimulus frequency. It is likely that several command fibres were stimulated

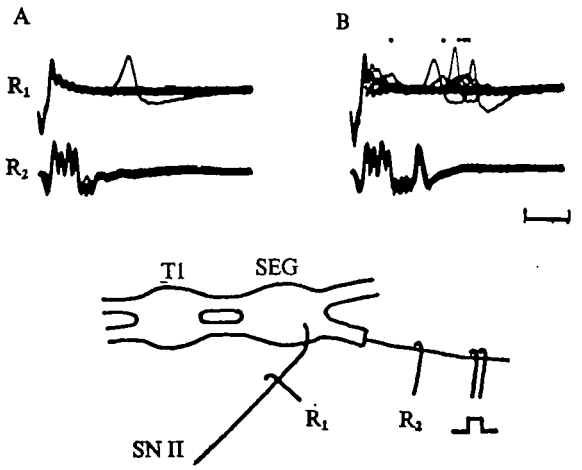

Fig. 3. Evidence that a single unit mediated command driven cardiac inhibition in one experiment. The cardioinhibitor nerve (SN II) was monitored by a suction electrode (R_1) while a single hook electrode (R_2) recorded evoked spikes in the active bundle teased from the connective. The records are multiple sweeps triggered by the stimulus delivered to the bundle containing a strong inhibitor unit. In (A) the stimulus voltage was subthreshold. A compound volley is seen in R_2, but no cardioinhibitor spikes are seen in R_1 (the large single spike was from a spontaneously firing motoneurone). In (B) the voltage was raised to threshold (4·8 V) and a single spike appeared in R_2 with a 3·8 msec latency. Simultaneously with the appearance of this spike, the cardioinhibitor neurone began firing (small spike marked by dots in R_1), and cardiac arrest occurred. Time base, 2 msec; T1 = first thoracic ganglion, SEG = subesophageal ganglion.

simultaneously in the latter instances. On occasion it was possible to separate the overt behaviour from the cardiac effect by further subdivision of the bundle, but at other times this was unsuccessful, suggesting that the two effects were mediated by the same unit.

Since notes were taken on evoked behaviours as each cardiac command fibre was characterized, it was possible to ask whether any behaviours were correlated with a particular type (inhibitor or accelerator) or locus of the cardiac units. We found the following motor patterns unique to inhibition: leg promotion ($N = 2$), abdominal extension ($N = 8$), abdominal flexion of segments 1 and 2 ($N = 2$), and uropod flaring ($N = 2$). Acceleration was uniquely accompanied by branchiostegite cleaning with the first two legs ($N = 6$), maxilliped beating ($N = 8$), leg and cheliped elevation and extension ($N = 13$), and cheliped flexion to the mouth ($N = 6$). In addition, the following occurred with acceleration in a high percentage of the cases: feeding (maxillipeds wiped legs and chelipeds, which flexed to mouth) (75%, $N = 8$), leg fanning (metachronal, coxal leg rotation) (79%, $N = 18$), leg flexion at MC joints (75%, $N = 8$), abdomen extension with swimmeret beating (72%, $N = 7$) or uropod flaring (75%, $N = 20$), abdomen flexion with uropod flaring (82%, $N = 11$). Among all of the above associations, only branchiostegite cleaning behaviour was clearly restricted to one locus (accelerator c.f. locus 9, Fig. 2B).

A question which immediately arises from the isolation and stimulation methodology employed is whether single units mediate the evoked changes being observed. Bowerman & Larimer (1974*a*), in addressing this problem, argued that (*a*) the presence of a sharp voltage threshold, (*b*) the repeatability of behavioural units and (*c*) the

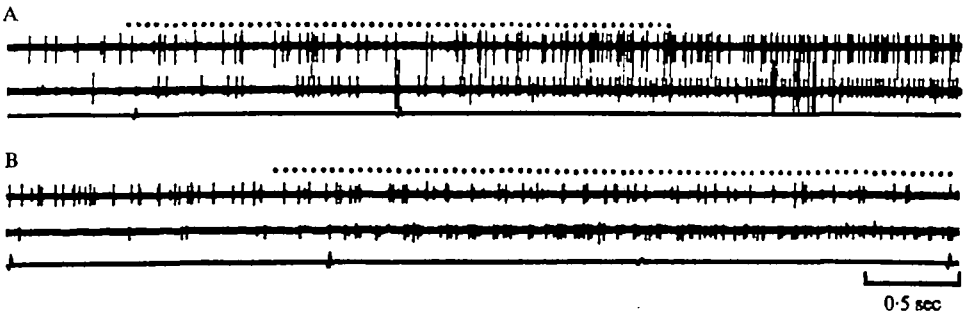

Fig. 4. The effects of command fibre drives on to the cardioregulatory neurones. (A) Record from the right cardioinhibitor nerve (top trace) and the left cardioinhibitor nerve (middle trace) during stimulation of a strong inhibitory command neurone at 20 Hz (indicated by dots above trace). Both cardioinhibitor neurones were driven into the characteristically prolonged burst mode; and cardiac arrest, indicated by the ECG cessation in the bottom trace, occurred midway through the record. Note the slightly stronger drive onto the contralateral (left) cardio-inhibitor neurone. (B) Record from the right cardioinhibitor nerve (top trace), the right cardioaccelerator nerve (middle trace) and the electrocardiogram (bottom trace) during 20 Hz stimulation (dots) of an accelerator command neurone. Firing of the tonically active cardioaccelerator neurone increased after a latency of about 500 msec; this was accompanied by a decrease in cardioinhibitor activity in the top trace. The acceleratory effect was rather weak, but consistent in this instance.

restricted occurrence of similar units to specific topographic loci in the connectives of many preparations provide indirect, yet substantial, evidence that single axons comprise the unit command fibre. Similar arguments may be brought to bear for the cardiac command fibres in the present study, although the 'motor score' (strong or weak inhibition or acceleration) is extremely simplified in this case. Direct proof that a single interneurone mediated the response was obtained on a few occasions. A recording electrode, R_3, was placed on the stimulated bundle, while another recording electrode, R_1, monitored the cardioinhibitor activity in SN II (Fig. 3). At a sub-threshold stimulating voltage a compound volley was evoked in the teased bundle (R_2) without resulting cardioinhibitor activity in R_1 (Fig. 3A); the single large spike in R_1 was a spontaneous motoneurone discharge. At a threshold of precisely 4·8 V, a single spike with constant latency (3·8 msec) appeared in the R_2 record (Fig. 3B, multiple sweep), and a concomitant cardioinhibitor discharge (small spikes indicated by dots) was recorded in R_1. A simultaneous monitoring of the ECG by the cardio-tachometer indicated that cardiac arrest occurred each time the R_2 spike appeared. It was concluded that this spike represented activity in the inhibitor command fibre because it occurred at the same threshold as the evoked inhibition.

The next phase of the present analysis involved examining the effects of cardiac command fibre drives onto the cardioregulatory neurones. Fig. 4A illustrates the effect of strong inhibitor stimulation on both the right (ipsilateral, top trace) and left (contralateral, middle trace) cardioinhibitor nerve activity. The 20 Hz stimulus is indicated by dots. An increase in cardioinhibitor neurone activity occurred within approximately 1 sec and was of a bursting nature. After 2 sec of stimulation the cardioinhibitors cascaded into their characteristic burst mode (Field & Larimer, 1974*a*) and cardiac arrest commenced (indicated by ECG record, bottom trace). As expected, the period of complete inhibition outlasted the stimulus duration. Attention should be drawn to the fact that the contralateral neurone, the left cardioinhibitor, began

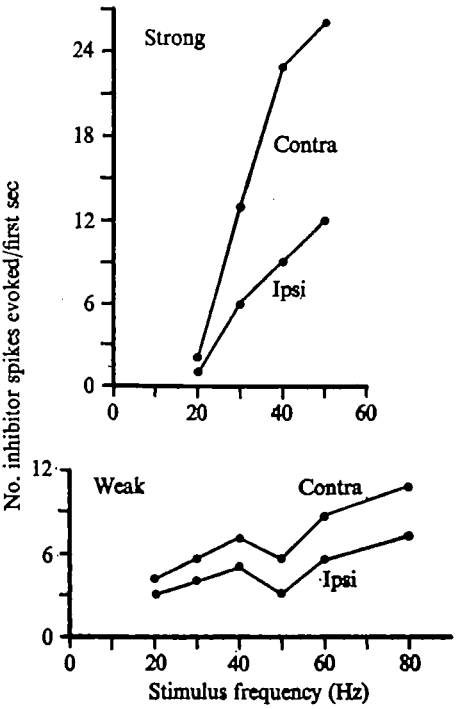

Fig. 5. Input–output plots of evoked cardioinhibitor activity versus frequency of command fibre stimulation. The top graph shows frequency–response curves for both the ispilateral and the contralateral cardioinhibitor neurones when driven by a strong inhibitor command unit. The steep slope of these curves contrasts with the shallow slope that characterized equivalent plots for a weak inhibitor command fibre (bottom graph). These results were consistent with plots for other inhibitor command units.

firing earlier and more actively than its ipsilateral counterpart. This phenomenon will be discussed more fully below. The effect of stimulating an accelerator command neurone is shown in Fig. 4B in a simultaneous recording from the ipsilateral cardioinhibitor nerve (SN II, top trace) and the ipsilateral cardioaccelerator nerve (SN III, middle trace). After approximately 500 msec the cardioaccelerator neurone (which fired at 2–3 Hz prior to stimulation) increased its discharge rate to near 20 Hz. Several other smaller units in the cardioaccelerator nerve also increased their discharge rate. The consequence of their activity is unknown. Fig. 4B also demonstrates a decrease in cardioinhibitor activity as the cardioaccelerator is selectively driven.

The bilateral effects of both weak and strong inhibitory command drives on to the cardioinhibitors was determined by recording from both SN II nerves while stimulating circumoesophageal units at various frequencies. Input–output plots of stimulus frequency versus evoked cardioinhibitor activity are shown in Fig. 5 for a strong (upper) and weak (lower) inhibitor command unit. The frequency–response curve for strong inhibitors had a steep positive slope while weak inhibitors had a shallow slope. Fig. 5 also clearly indicates the previously mentioned property of stronger command drive on to the contralateral, relative to the ipsilateral cardioinhibitor. For this analysis 16 inhibitory command fibres were tested 51 times at various stimulus frequencies; every test yielded a stronger contralateral drive. Cross-connectivity was also noted by Wiersma & Novitski (1942) for the inhibitory fibre which they examined.

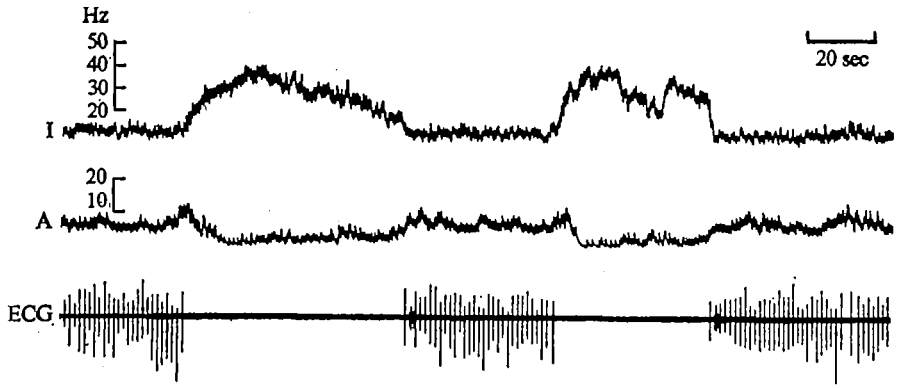

Fig. 6. An example of reciprocity between the cardioinhibitor and cardioaccelerator neurones. The top trace gives the firing frequency of the cardioinhibitor (I) and the middle trace shows the cardioaccelerator firing frequency (A). The ECG is shown in the bottom trace. A strong inhibitory command fibre was stimulated (8·4 V, 10 Hz) for about 3 sec to produce the first period of cardiac arrest. The second period of inhibition occurred spontaneously. Both show a decrease in accelerator discharge as the inhibitor increased its firing rate.

Reciprocity between the cardioinhibitor and cardioaccelerator neurones (in SN II and SN III) was mentioned briefly in the preceding paper (Field & Larimer 1974*a*) with reference to a decrease in cardioaccelerator activity during the glucose inhibitory reflex. In the present paper it was shown that cardioinhibitor activity decreased when an accelerator command fibre was stimulated (Fig. 4B). The opposite effect, a decrease in cardioaccelerator firing rate when an inhibitor command fibre is driven, is demonstrated in Fig 6. The d.c. analogue of firing frequency was recorded for the ipsilateral cardioinhibitor (top trace) and cardioaccelerator (middle trace); the bottom trace, showing the ECG, indicates when cardiac arrest occurred. During the first period of inhibition (left side) a strong inhibitor command unit was stimulated at 10 Hz and 8·4 V for about 3 sec. As the cardioinhibitor reached a maximum firing rate of almost 40 Hz, the cardioaccelerator rate dropped from a pre-stimulus level of approximately 7 Hz to a minimum of 1 Hz. A return to the original cardioaccelerator frequency accompanied the drop in cardioinhibitor spike frequency. Shortly after this bout a spontaneous period of cardiac arrest occurred (right side of record) and the same reciprocity features were displayed. In the case where no command fibres were activated, i.e. the glucose inhibitory reflex (Fig. 6; Field & Larimer, 1974*a*) a similar pattern of reciprocity was observed.

DISCUSSION

In this report we have extended the analysis of cardiac control to include interneurones in the circumoesophageal commissures which mediate inhibition and acceleration of the heart. Although we characterized a surprisingly large number of 'cardiac command fibres', more exist in other parts of the CNS, since Wiersma & Novitski (1942) reported cardiac effects upon stimulation of the abdominal nerve cord.

Function. In view of their abundance, it is appropriate to ask how these interneurones might function in the intact animal. A number of reasonable explanations arise, the most obvious of which is that they provide a descending route for sensory information from visual, chemical and tactile receptors in the cephalic region. This

hypothesis is supported by our previous report that stimulation of the antennal, antennular and tegumentary nerves evoked inhibitory cardiac reflexes (Field & Larimer, 1974*a*); in addition we reviewed evidence (Larimer & Tindel, 1966) for a visual influence on the heart rate. Another potential capacity served by the commissural interneurones could be to transmit circadial regulatory commands descending from the brain to the heart. Circadian cardiac rhythms have been described, although their mode of regulation (neural or humoral) remains unknown (Maynard, 1960). Rather than representing a set of descending pathways, a third possible function may involve the transmission of efferent copy signals to the brain from cardiac reflex circuits in the suboesophageal ganglion during periods of cardiac regulation. Cardiac interneurones may also represent (at least in part) the well-known command fibres which mediate overt behaviour patterns (Atwood & Wiersma, 1967; Evoy & Kennedy, 1967; Bowerman & Larimer, 1974*a*, *b*). Cardiac changes evidently have not been monitored in previous investigations of behavioural command fibres, yet there is no reason to negate the possibility that such changes may accompany stereotyped behaviours. Evidence from the present study provides some support for this concept, particularly the observations that certain teased axon bundles drove both cardiac effects and behaviour patterns at identical thresholds and similar frequency responses (Table 4). Furthermore, a number of behavioural patterns were shown to be uniquely or primarily associated with either cardiac accelerator or cardiac inhibitor command units.

In view of the diversity and number of cardiac command fibres, it would seem likely that they could function in combination with each other and in conjunction with other commands. The low voltage threshold of some of the units may indicate that relatively large axons are responsible for the phenomena reported here. If true, intracellular studies of these command units are likely to provide answers to the problem of how these units function in the crayfish.

Connectivity. Since both cardioinhibitor regulatory neurones converge upon a single target organ, however, it was surprising to find that command interneurones often affect them differentially. Asymmetric drive by command fibres has been described for control of the crayfish uropods (Larimer & Kennedy, 1969), but this result was to be expected owing to the animal's utilization of asymmetrical uropod postures for steering during locomotion (see also Bowerman & Larimer (1974*a*) on command-driven turning behaviour). Cardiac inhibitor command neurones preferentially activate the contralateral cardioinhibitor neurone, but thoracic sensory input (tactile, glucose receptors) excites the ipsilateral cardioinhibitor more strongly (personal observation). This suggests that either there is a circuit which receives command fibre and thoracic sensory input and excites both cardioinhibitors via collaterals, or that the command fibres and thoracic afferents themselves have parallel bilateral (but unequal) input directly onto the cardioinhibitors. It was previously shown that excitation of both cardioinhibitors by unilateral stimulation is not due to direct excitatory coupling between cardioinhibitors (Field & Larimer, 1974*a*). Intracellular dye injection experiments should help to resolve this question by revealing collaterals responsible for contralateral cardioinhibitor excitation.

Although accelerator–inhibitor reciprocity was demonstrated by stimulation of cardiac command fibres, this property is not likely to be inherent in the command units themselves. This conclusion is drawn from the observation that reciprocity was

observed during chemical and tactile reflex inhibition of the heart, which would probably not involve activation of the command neurones (unless they were providing efference copy from the suboesophageal ganglion to the brain). Instead, reciprocity is more likely to be a property of the cardioregulatory neurones or interneurones presynaptic upon them in the suboesophageal ganglion.

It is perhaps reasonable to adopt the idea of McMahon & Wilkens (1972) in postulating a central integrating circuit which receives all the appropriate afferent input and activates cardioinhibitors or cardioaccelerators accordingly. In the lobster *Homarus*, McMahon & Wilkens (1972) have shown that the scaphognathite beat is simultaneously inhibited during spontaneous cardiac inhibition, thus emphasizing the probability of a central control area that mediates circulatory and respiratory function. The degree to which the cardioregulatory neurones comprise such an integrating control centre remains to be elucidated.

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