J. Exp. Biol. (1974), 60, 119–134
With 5 text-figures
Printed in Great Britain

COMMAND FIBRES IN THE CIRCUMOESOPHAGEAL CONNECTIVES OF CRAYFISH

II. PHASIC FIBRES

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(*Received* 25 *June* 1973)

INTRODUCTION

Interneurons capable of evoking some of the more complex behaviour patterns of crayfish have axons that course through the circumoesophageal connectives (Atwood & Wiersma, 1967). A survey of the positional behaviour patterns that can be driven by fine-bundle stimulation at this level has been presented in a previous paper (Bowerman & Larimer, 1973). The current report extends the survey to include the major phasic and cyclical drives such as forward and backward walking, escape or swimming activity as well as some less well defined behaviour patterns resembling cleaning and feeding.

Phasic and cyclical movements are of special interest because each can be more readily assigned to the normal repertory of the animal than can many of the slow more limited movements or those that effect a positional change. This not only facilitates to some degree the establishment of identities among interneurons in different individuals, but offers the opportunity for comparison of command-fibre-driven behaviour patterns with those generated by normally behaving animals (Larimer & Eggleston, 1971; Wine & Krasne, 1972). In addition, knowledge of command fibres may allow an important behaviour pattern to be experimentally controlled, even if it is seldom used by the animal. A behaviour pattern such as swimming is of interest for several reasons. Its execution, for example, demands extensive and rapid co-ordination throughout the central nervous system, suggesting that a hierarchy of precisely coupled neurons exist to accomplish it. Fast tail-flips also utilize the phasic extensor and flexor muscles of the abdomen, and until now no identified interneurons have been described that activate the fast abdominal extensor motor neurons, and relatively few (other than the giant fibres) that mediate fast flexion. It was felt, therefore, that a study of these behaviour patterns would be advanced if additional knowledge of their underlying command interneurons was generally available.

MATERIALS AND METHODS

The crayfish, *Procambarus clarkii* (Girard) was used throughout the experiments. Each individual was secured by the left branchiostegite to the end of a rigid Plexiglass rod using epoxy glue. The circumoesophageal connectives were exposed by cutting

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a window anterior to the cephalic groove and removing the stomach and hepat[illegible] pancreas. After flushing the cavity thoroughly, the preparation was immersed in saline (van Harreveld, 1936) maintained at 12–15 °C. Survival of the preparation was further enhanced by ligating the arteries to the brain and by elevating the oxygen tension of the saline (Bowerman & Larimer, 1973). The animal was positioned over the outer edge of a freely rotating horizontal walking wheel which provided more normal proprioceptive feedback and stepping sequences than an immobile platform.

The right circumoesophageal connective was de-sheathed, and the giant fibres were removed. Both connectives were crushed near the brain thus obviating potentially disruptive neural influences. Small bundles of fibres were stripped from the connective and suspended on hook electrodes for stimulation.

Behavioural output was filmed at 8 frames/sec from the side or from above with a Beaulieu 16 mm camera on Kodak Tri-X film. The command-fibre number, stimulation frequency and stimulus monitor light were all recorded directly on the cine film. The approximate position of each nerve bundle was marked on a cross-sectional map of the circumoesophageal connective (Wiersma, 1958).

RESULTS

As noted above, the various outputs evoked by phasic command fibres are somewhat more amenable to the establishment of identities than are all but the most graphic tonic drives. We have therefore attempted to establish the types of phasic drives present and to decipher those that appeared to be identical among the group. Several criteria have been utilized in an effort to verify that a behaviour pattern was evoked by stimulation of a single interneuron. These include the constancy of threshold voltage for evoking an output, the behavioural repeatability within an individual and among individuals, and the distribution of axon locations within the connective (Bowerman & Larimer, 1973).

The command fibres providing for phasic behaviour patterns have been divided into three major classes: those which trigger forward walking, backward walking and isolated tail-flips or swimming. Several minor groups are also recognized and described briefly.

Figures illustrating evoked behaviour patterns were prepared from outline tracings of projected still frames. Although static presentations of this type result in a major loss of behavioural detail, they offer the best means available to reduce the data into a presentable form.

Forward walking

A total of 22 command interneurons have been examined which evoked forward walking (Table 1). All of these were obtained from animals that were provided with a walking wheel, with a maximum of four forward walking interneurons found in one individual (animal 24). An examination of axon locations for the forward-walking fibres (Fig. 1) shows that they fall into three loose clusters, corresponding to areas 64–66, 68–69, and 70–72. In several individuals (animals 20, 24, 28), forward-walking fibres have been found in each of these areas. Otherwise, when only one or two forward-walking fibres were found in a single preparation, they too fell within these groups with the exceptions of fibre 570 in area 74 and fibre 461 in area 62.

Table 1. *A summary of command interneurons effective in generating forward-walking behaviour*

(Command-fibre numbers are listed together with the preparation number from which each was obtained and the identity to which the element was assigned. Identities 1 to 5 inclusive comprise corresponding interneurons from different animals on the basis of similarities of axon location and evoked behaviour as presented in Fig. 1.)

C.F.	Animal	Identity	C.F.	Animal	Identity
370	16	3	469	23	1
			473	23	2
387	17	1			
			565	24	1
397	18	1	570	24	4
405	18	3	572	24	3
			582	24	2
414	19	1			
418	19	2	601	25	2
			608	25	3
426	20	1			
437	20	2	620	26	1
441	20	3			
			637	28	1
461	22	5	644	28	2
			651	28	3

Prior to the introduction of the walking wheel, two interneurons were found in area 74 near the location of fibre 570 that may well have been the corresponding interneurons in those preparations. On the basis of these considerations we have concluded tentatively that at least five command interneurons are present at the level of the circumoesophageal connectives that are capable of evoking forward walking. Establishment of command-fibre identities among the interneurons within the forward-walking class proved difficult due to the similarity of the various evoked outputs. Each command element generated elevation of the chelipeds and abdominal extension. There were some differences in the speed of walking but this did not appear constant for fibres within a proposed identity. The criterion of axon location was also inconclusive, with the scatter probably reflecting inaccuracies in estimating the positions of axons within the map areas.

The latency between stimulus onset and initiation of leg movements proved quite variable. For example, data taken on command element 608 showed an inverse relationship between stimulus frequency and latency to onset of walking. Here, walking was initiated only after 2 sec of stimulation at 50/sec. When the input frequency was elevated to 75/sec, the latency was lowered to 1 sec, and at 100/sec it was further reduced to *ca.* $\frac{1}{2}$ sec. In most instances forward walking was abruptly terminated when stimulation stopped; in a few cases, however, it continued for several seconds.

Stepping sequences generated by the stimulation of a command interneuron are given in Fig. 2A. These data were derived from film analysis of ipsilateral limb movements (animal 25, fibre 608, group FW3). The frame in which an appendage was lifted, i.e. the end of the powerstroke, was chosen as a reference point for the stepping cycle. The important features to be noted are that both the stepping sequence and stepping frequency remain regular for the locomotory appendages throughout the period of the stimulation. Upon cessation of the command drive, evoked output was

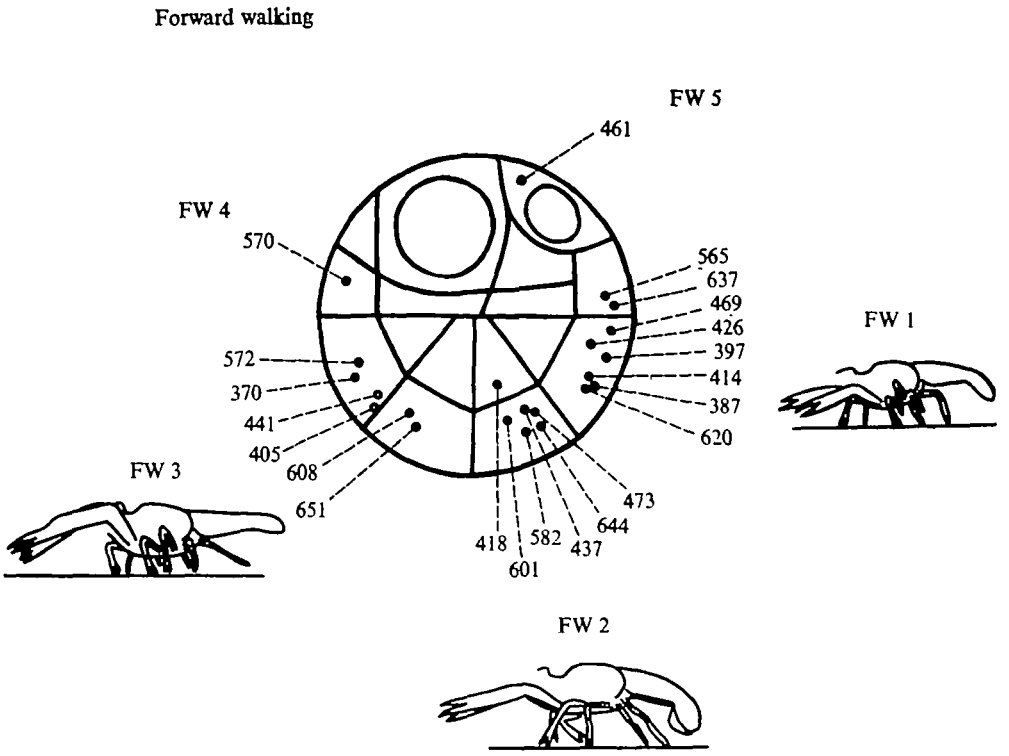

Fig. 1. Forward-walking command fibres. The positions of 22 command-fibre axons that evoked forward walking are noted on a cross-sectional map of the right circumoesophageal connective. As noted in the text, on the basis of location and preparation number, the command elements have been subdivided into five putative identities: FW 1, FW 2, FW 3, FW 4, and FW 5. Profile drawings are presented for representative units from the three best established identities to illustrate positional components of the evoked behaviour. Ipsilateral walking legs contributed to the turning of the walking wheel by remoting in the depressed, powerstroke position and promoting in the elevated attitude. The contralateral walking legs often stepped at a reduced frequency and sometimes exhibited a lower level of overall coordination than the ipsilateral legs. This was attributed to the differential radii that the two sides experienced on the walking wheel.

rapidly terminated. In several instances where limb segments were missing and the terminal aspect of the appendage was not in contact with the walking wheel, relatively normal stepping sequences were maintained. It should be emphasized that Fig. 2 is merely an attempt to illustrate the co-ordinated nature of command-fibre-evoked walking in lieu of direct inspection of the original cinematographic data.

Backward walking

Some 24 command fibres were found which were capable of evoking backward walking in conjunction with a variety of cheliped and abdominal positions (Fig. 3). A summary of command-fibre number, animal or preparation number and the identity to which a fibre was assigned, is presented in Table 2. As was the case for forward-walking command elements, no backward-walking drives were noticed prior to the introduction of the walking wheel. Subsequent to animal 14, all preparations except two contributed at least one backward-walking command fibre. The co-ordinated

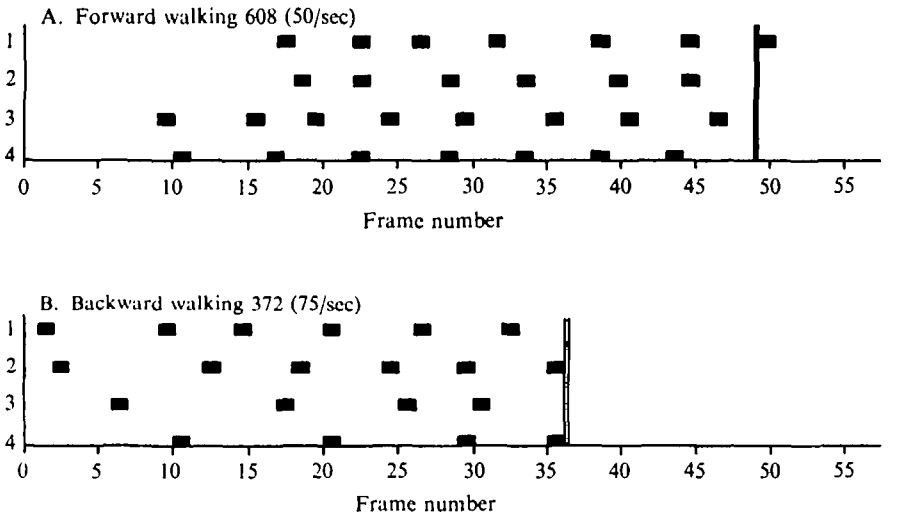

Fig. 2. A. This plot, which has been generated from cinematographic data, displays the co-ordinated nature of the stepping sequences evoked by forward-walking command fibres. Data are given for ipsilateral walking legs 1 to 4 inclusive resulting from stimulation of interneuron 608 at 50/sec. Frame numbers subsequent to the initiation of command-fibre stimulation are noted on the abscissa. This information can be converted into real time by taking into account the filming speed of 8 FPS. Stimulus cessation is marked by the vertical bar. The bars, which serve as reference points within the stepping cycles, denote the frame number in which an appendage was elevated at the termination of the powerstroke. B. The organization of Fig. 2B is identical to that of 2A, but in this case the backward-stepping pattern was generated by stimulation of backward-walking command fibre 372 at 75/sec.

Table 2. *A summary of the command interneurons which were effective in evoking backward-walking behaviour*

(The command fibres are listed along with the animal from which they were obtained, and classified into four identities. Each group is believed to be made up of corresponding interneurons on the basis of similarity of axon location and evoked behaviour, as presented in Fig. 3. A fifth, miscellaneous group of fibres (not an identity) that release backward walking is also given, as shown in Fig. 4. (See text.))

C.F.	Animal	Identity	C.F.	Animal	Identity
361	15	5	432	20	1
			435	20	5
368	16	3			
371	16	5	453	21	2
372	16	1			
			465	22	5
385	17	4			
388	17	2	467	23	3
			470	23	2
402	18	2	478	23	5
409	19	1	597	25	5
411	19	2			
419	19	4	615	26	5
			617	26	2
426	20	2			
429	20	5	638	28	1
			640	28	3

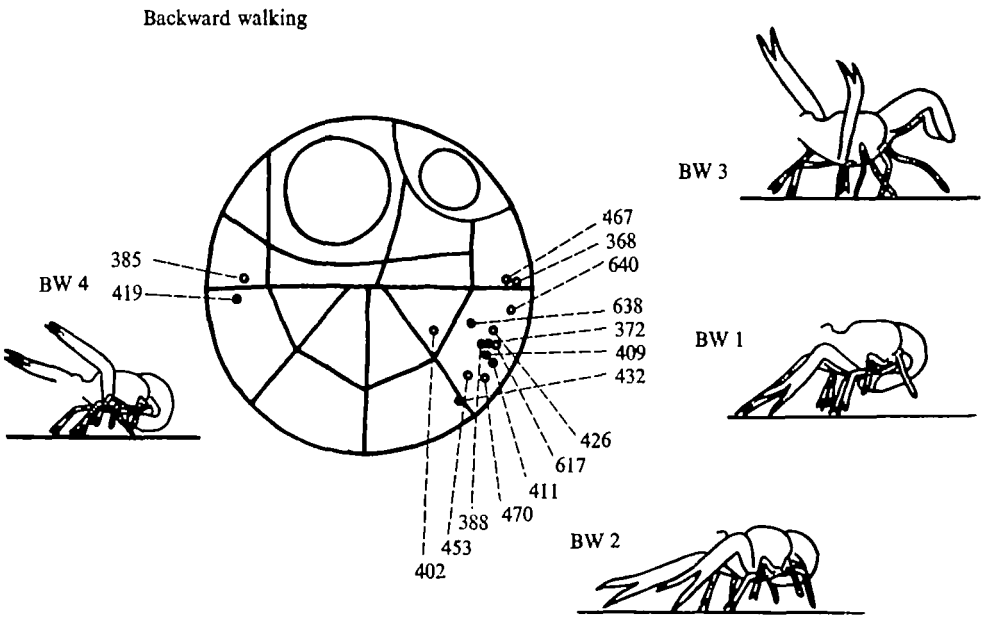

Fig. 3. Backward-walking command fibres. The location of 16 command fibres that evoked backward-walking are given. On the basis of cross-sectional location, preparation number, and cheliped and abdomen position these units have been subdivided into four putative identities; BW 1, BW 2, BW 3, and BW 4. Two of these, BW 1 and BW 2, are similar both in evoked behaviour and location within the connective. They were ultimately separated, perhaps artificially, on the basis of their apparent efficacy at evoking movement. The three units which comprise BW 3 evoke a stereotyped cheliped and abdominal geometry. The rate of backward walking generated by stimulation of BS 3 units, however, was comparatively low. The two units assigned to the BW 4 identity, as shown in the profile drawing, also evoked bilateral cheliped lifting but (unlike BW 3) BW 4 evoked abdominal flexion, together with low-level backward walking.

nature of the walking activity for the ipsilateral appendages is demonstrated by stepping data extracted from movements commanded by stimulation of BW element 372 at 75/sec (Fig. 2B). For each walking leg the frame number in which the appendage was lifted (end of powerstroke) is noted. Latency between stimulus onset and initiation of the walking programme was short, being only one frame (125 msec). Activity was generally terminated abruptly upon cessation of command stimulation. As noted for Fig. 2A, the stepping data for backward walking are not to be construed as representing a quantitative evaluation of evoked behaviour, but instead should be viewed as an illustration of the degree of coordination of involved appendages.

Specific features of the backward-walking programmes evoked by different identities are variable, and this aided in their subsequent classification into distinct groups, denoted as BW 1–4 (Fig. 3).

Command fibres which comprise the BW 1 identity have been found four times (Fig. 3). When a BW 1 unit was stimulated at 75/sec, the abdomen was tightly flexed under the cephalothorax and the walking legs stepped so as to spin the wheel forward under the animal. This was accomplished by cyclical activity in which remotion and elevation occurred together as did promotion and depression. The chelipeds were operative at a reduced frequency, and appeared to contribute to the locomotory effort

However, myograms from the appropriate muscles would be needed to eliminate the possibility that the chelipeds were passively extended by the rotating wheel and then merely repositioned by flexion. The BW1 elements generated more rapid locomotion than any other BW identity, with cycle times for individual appendages being generally less than 1 sec.

The 7 fibres which have been grouped as a BW2 identity are located in the same area within the connective as are the BW1 units (Fig. 3). Activity evoked by BW2 elements consisted of maximal abdominal flexion coupled with backwards locomotion. This was performed by all of the walking legs (Fig. 3). As was the case for BW1, however, the contribution of chelipeds to the actual generation of locomotor forces remained equivocal. An apparent feature of this group was that the stepping rates generated by BW2 units were slower than those generated by BW1 fibres. The separation of the backward-walking command fibres from this area of the cord into two identities must remain somewhat tenuous considering their general similarities. Nonetheless, this subdivision was deemed necessary, since several preparations contributed two such backward-walking elements from nearly identical cord locations.

The unique bilateral cheliped and abdominal positions evoked by stimulation of command fibres within the BW3 identity are presented in Fig. 3. This overall configuration was reminiscent of a defence posture, with the chelipeds bilaterally lifted and extended, the chelae open and the abdomen extended. With stimulation frequencies of 50 or 75/sec low-level backwards walking was also elicited. As noted in the preceding paper (Bowerman & Larimer, 1973, Fig. 5), this total body geometry can be attained at the lower stimulation frequency of 20/sec without the recruitment of the backward-walking programme.

Several backward-walking command fibres, e.g. 385 and 419, have been found in the midmedial aspect of the circum oesophageal connective (Fig. 3). These were the only elements within the backward-walking classification found in this area and were, partially on this basis, assigned to identity BW4. Evoked behaviour consisted of abdominal flexion and bilateral lifting of the chelipeds. As with the BW3 group these fibres provided only a low-level backwards-walking drive.

The remaining backward-walking command fibres could not be assigned to any particular BW identity and are grouped in the miscellaneous category. Examination of the locations of these various elements (Fig. 4) indicates that six of the eight were found in those areas of the connective which provided the great majority of the identified backward-walking command neurons. Several of these miscellaneous elements may be identical to fibres in the BW1–4 groups, but when stimulated had provided an incomplete output, or evoked a confused behaviour pattern due to contamination of the bundles with other command fibres.

Escape and swimming

Command fibres in the escape or swimming categories share the feature of being capable of evoking rapid abdominal movements. The command-fibre number, animal number and identity number are presented for all these elements in Table 3. Fig. 5 presents outlines for representatives of each of the five command-fibre identities, as well as their axonal locations within the circumoesophageal connective.

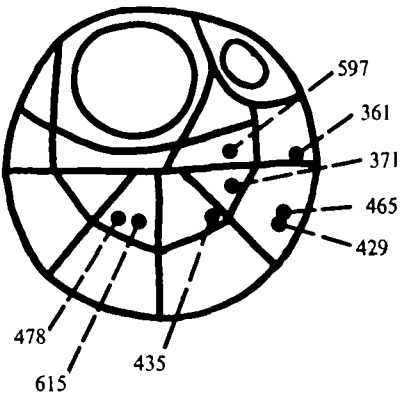

Fig. 4. Miscellaneous: backward-walking command fibres. Certain command fibres which evoke backward walking could not be assigned to an established BW identity or organized into new ones. The spacial distribution of these miscellaneous elements is shown on the cross-sectional map.

Subsequent to the onset of command-fibre stimulation, the characteristic rapid tail-flip or flips were evoked at a wide range of latencies. In addition, upon repeated trains of stimulation, tail-flips were expressed only a portion of the time. Nevertheless, when the rapid movement did occur, it appeared as the stereotyped abdominal movement representative of that specific identity. In these respects the swimming command fibres are different from other command elements which evoke their characteristic outputs in a constant, less-variable fashion.

The 4 command fibres comprising the S1 identity (Fig. 5) were, of all command elements, the most capable of evoking a maintained sequence of tail-flips. Fibre 350 generated rapid and maintained swimming and is discussed as representative of the identity. The latency between stimulus onset (75/sec) and initiation of appendage and abdominal movements was less than one frame (125 msec). With maintained stimulation of C.F. 350, over 3 sec of strong swimming comprising at least 24 flexion-extension cycles occurred. As noted by the S1 profiles presented in Fig. 5, rapid extension positioned the abdomen in an elevated geometry, while the powerstroke component of the cycle brought the abdomen to a well-flexed position. Subsequent to the cessation of stimulation, rapid tail-flip activity was terminated within 2 frames (250 msec). The walking legs and chelipeds were affected by stimulation of S1 fibres, but not in an easily characterized manner. Their output may be best described as rapid and unco-ordinated. It was evident, however, that these appendages were not positioned in a streamlining posture.

Command fibres assigned to the S2 identity generated abdominal movements which were performed at a relatively extended position (Fig. 5). The activation was initiated by attainment of a relatively extended abdominal position accompanied by swimmeret beating. This extension was not particularly rapid since, as noted by the top S2 profile (Fig. 5), it took 2 frames for completion. At no time during the subsequent several cycles of movement was the abdomen flexed under the cephalothorax. As with the S1 group, the appendages were influenced in a relatively non-specific fashion by these command fibres. A tendency to lift the ipsilateral walking legs wa

Table 3. *Information concerning the tail-flip and swimming command fibres*

(Command-fibre number, animal or preparation number, and identity number for the various elements are presented in chronological order.)

C.F.	Animal	Identity	C.F.	Animal	Identity
154	3	5	365	15	4
174	3	3			
			375	16	1
238	8	2			
			419	19	4
244	9	5			
262	9	3	444	20	2
306	11	4	480	23	3
329	13	2	585	24	1
337	13	3			
			600	25	1
350	14	1			

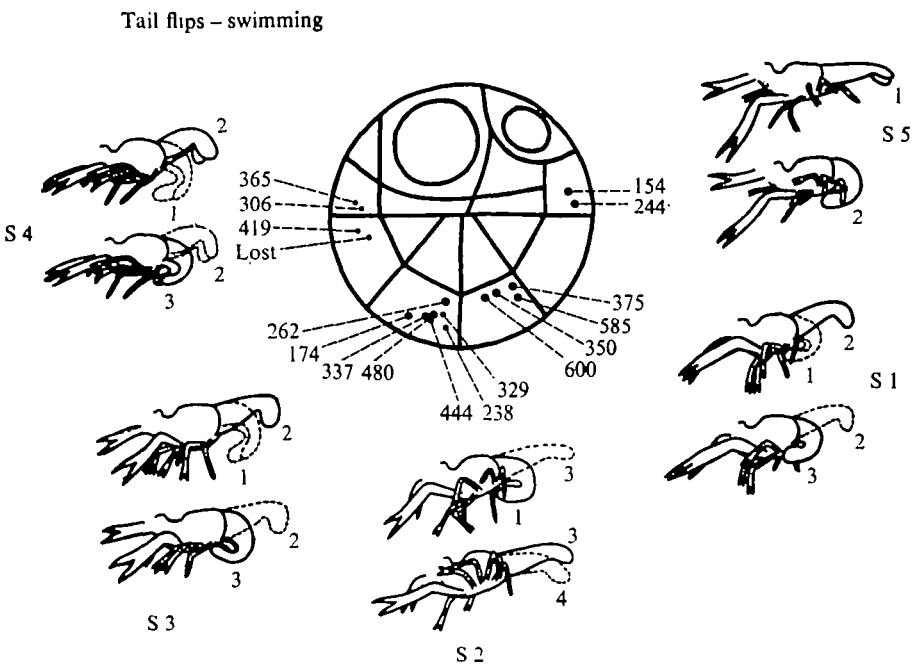

Fig. 5. Escape-swimming command fibres. Each command fibre that evoked tail-flips or swimming activity has been assigned to one of five identities. The location and number of the command element, together with several representative illustrations from each identity, are displayed. Since temporal considerations were of major importance for characterizing these elements, frame-number information (at 8 FPS) is presented adjacent to each outline.

observed although other, less definable movements occurred as well. No streamlining effect was present.

Four command fibres were isolated from the circumoesophageal connective which exhibited enough behavioural similarity and axonal proximity to be grouped as the S 3 identity (Fig. 5). Ongoing stimulation of these command fibres resulted in the generation of isolated tail-flips at quite variable latencies. With C.F. 174, for example,

there was a 7 frame latency prior to the initial tail-flip and an additional 11 frames until the next. During a subsequent stimulation sequence, the delay interval was 29 frames. The tail-flip cycle was initiated from a loosely flexed abdominal position (Fig. 5, S3 outline). Extension, which also proved variable in extent for the different S3 command elements, was completed in less than 125 msec, i.e. between frames 1 and 2. Relatively tight abdominal flexion (frame 3 of the cycle) invariably followed fast extension in the dozen or so cycles evoked by stimulation of an S3 unit. This suggests that the two components of the tail-flip cycle are coupled by means of a central programme or by some unknown proprioceptive reflex. The chelipeds and walking legs were extended and promoted during the S3-evoked behavioural programme to accompany the flexion component (Fig. 5, S3 profile). Such streamlining may augment the effectiveness of any backwards propulsive force generated by the fast abdominal flexion.

Command fibres assigned to the S4 identity were found four times, with one of these being lost prior to the photographing of its evoked output (Fig. 5). As was the case for other swimming command elements, the latency between stimulus onset and occurrence of fast abdominal movements was relatively long and variable. Latency values of 13 frames for C.F. 365 and 10 frames for C.F. 419 were recorded. The specifics of abdominal movement are noted by the S4 profiles presented in Fig. 5. Extension from the initially flexed abdominal geometry and the flexion powerstroke each required one frame for completion. The S4 command elements were capable of generating continuous sequences of two or three tail-flip cycles and should therefore be considered as evoking swimming instead of simple escape. As was the case for the S3 elements, these fibres evoked bilateral promotion and extension of the appendages, with the streamlining effect being evident prior to the actual initiation of the fast abdominal movements.

Two elements (154 and 244) which comprise the S5 identity are different from all other tail-flip and swimming command fibres in that they evoked slow abdominal extension followed by a single phasic flexion (Fig. 5, S5 profile). S5 command fibres were the only command elements which evoked only half of a tail-flip cycle, specifically fast flexion. There was no streamlining posture generated in conjunction with the escape behaviour.

Miscellaneous command fibres

Other command-fibre drives were encountered in addition to those evoking the walking and escape. One class of such units was differentiated from other phasic drives in terms of the apparent low-level of co-ordination. The behaviour pattern involved rapid bilateral 'searching' activity of the chelipeds and first two pairs of chelate walking legs in addition to movement and orientation of these appendages about the oral region. Hence, these elements were termed 'feeding fibres'.

Another class of command fibres evoked scratching of the ipsilateral branchiostegite by the first two or three walking legs and occasionally by the cheliped. Upon electrical stimulation appendages were lifted and rotated anteriorly, thus bringing them in contact with the branchiostegite. Subsequent promotion-remotion movements appeared to effect scratching of the lateral branchiostegite surface.

Intact crayfish can utilize the fourth pair of walking legs to clean the dorsal aspect

of the abdomen. During this behaviour pattern the appendage is rotated and elevated over the flexed abdomen and subsequently rubbed back and forth. Behaviour quite similar to this was also generated as a consequence of stimulation of several command fibres.

The last class of cyclical command output to be described resembled a behaviour pattern of unknown function that is frequently observed in intact crayfish. This behaviour pattern involved slow alternating promotion-remotion cycles of the walking legs in an otherwise quiescent animal. During this activity the appendages were maintained on the substrate in a depressed mode.

DISCUSSION

The variety of movements which were evoked by stimulation of circumoesophageal command fibres proved especially interesting by virtue of their similarity to spontaneous behaviour patterns observed in intact animals. The reproducibility of experimentally generated output, in conjunction with voltage threshold and axon location, have established multiple identities for the major command-fibre classifications. However difficult it is to provide some sort of functional interpretation for the majority of tonic command fibre outputs, the significance of phasic command output appears clear-cut. It is postulated that these command elements are in fact utilized by the crayfish for the performance of the corresponding behaviour patterns.

The data provide strong indications that command elements and the associated driving-distributing neural networks are organized to provide for partial or even total behaviour patterns. This is in essence supportive of the ideas of Hughlings Jackson who, in the last century, considered aspects of cerebral cortex organization in mammals. At that time he proposed that the basic organization of the cerebral cortex was along the lines of providing for movements rather than the activation of discrete muscles (see discussion by Evarts, 1967).

The circumoesophageal command-fibre experimentation has re-emphasized certain questions which may prove quite difficult to answer. For example, it is not known whether command fibres within a basic classification (e.g. forward walking) are used singly or in ensembles. If used in isolation, what are the critical integrative pathways resulting in the decision to activate one behavioural switch over another? On the other hand, if neural ensembles are utilized, how are the various decision-making circuits established so as to incorporate the desired complex interneuronal outputs? Experiments focusing on these questions would require at the very least direct electrophysiological monitoring of characterized command fibres in a free-moving crayfish in conjunction with the ability to electrically stimulate the putative command elements which express the corresponding behaviour pattern. Conceivably with this procedure one could illustrate the simultaneous utilization of two or more command fibres of a discrete class for a behavioural output. An unequivocal demonstration of an act of behaviour as a consequence of single command-fibre activity would be unlikely, however, since other elements may have been merely overlooked.

T. Page (personal communication) has attempted to deal with certain of these questions by using tungsten electrodes implanted in the circumoesophageal connectives of unrestrained crayfish. While it was possible to characterize a wide array of

sensory interneurons, similar to those previously described in dissected preparation (Wiersma & Mill, 1965), evidence that activity in command interneurons was responsible for generating behaviour patterns was not discovered. Interneuronal activity (not sensory in origin) could be recorded in association with various behavioural efforts by the crayfish, but attempts to elicit the same behavioural output by stimulation through the implanted electrode were unsuccessful. A variety of interpretations of these results are possible. One such explanation involves the concept of central excitatory state. If lower ganglionic centres are under strict descending supra- and suboesophageal control, the imposition of activity in a single specific command element, driven out of context by the experimenter, may simply be ignored. Experience from the current study clearly suggested that elimination of the supraoesophageal ganglion enhanced the behavioural expression of circumoesophageal command fibres. Questions dealing with the input side of command-fibre systems are even more difficult to approach and may have to wait for more background work not only on specific command-fibre pathways, but on integrative phenomena in general.

As noted above, it was not possible to differentiate clearly between the various outputs obtained from the forward-walking command fibres due to their general similarities. In earlier command-fibre experiments, neuronal components which were initially considered functionally redundant were subsequently identified as unique entities. The medial and lateral giants, for example, ultimately proved to be quite dissimilar in terms of specific excitatory inputs as well as in terms of behavioural output (Larimer *et al.* 1971; Wine & Krasne, 1972). From command-fibre experiments performed on the abdominal cord it was noted that extension and flexion elements which superficially formed homogeneous classes, differed in terms of specific motor neuron recruitment profiles within a segment and in the spacially distributed efficacy over several segments (Evoy & Kennedy, 1967; Kennedy *et al.* 1967). Since an apparent redundancy may instead reflect an incomplete understanding, groups of command fibres, such as those evoking forward walking, should perhaps be viewed as displaying similarity instead of redundancy.

Walking

During experiments in which the crayfish were either freely suspended or supported by an immobile platform (preparations 1–14), there were only hints of evoked backward-walking or forward-walking behaviour. Based on the knowledge that proprioceptive feedback has been implicated in arthropod appendage movement and posture control systems in crustaceans (Evoy & Cohen, 1969; Barnes, Spirito & Evoy, 1972; Davis, 1969*a*; Paul, 1971), in insects (Wilson 1961, Runion & Usherwood, 1968; Usherwood, Runion & Campbell, 1968; Pearson, 1972), and in arachnids (Land, 1972), a walking wheel was supplied for the preparation. It should be noted that Atwood & Wiersma (1967) also suggested that proprioceptive feedback might well be required for expression of command-fibre-evoked locomotory behaviour. In preparations immediately subsequent to the improvisation of the walking wheel the existence of command fibres that evoked walking was revealed. This could be taken as evidence for the contribution of sensory feedback in either a tonic excitatory mode (Wilson, 1961; Pearson, 1972) or in a more complex motor-shaping capacity (Runion & Usherwood, 1968; Davis, 1969*a*; Paul, 1971; Evoy & Cohen, 1969). It may als

forecast experimental difficulty in analysing the walking oscillators electrophysiologically if in fact central neural components require specific spatially and temporally organized afferent feedback, generated as a consequence of co-ordinated appendage activity.

Although the walking wheel certainly permitted the behavioural expression of the various locomotory command elements, it was not ideal in every respect. Ipsilateral walking legs near the periphery of the wheel stepped a greater distance per revolution than did the contralateral legs. Such asymmetry probably contributed a bilateral irregularity of stepping patterns. Also, even though the walking wheel was provided with a rough surface, the appendages seemed to slip. This too may well have affected the expression of the command-fibre-evoked walking activity. Other deficiencies of the walking programme could have resulted from the fixed elevation of the preparation and inadequacies involving loading and wheel inertia. In spite of such experimental imperfections it appears well established that command fibres evoking forward and backward walking have been demonstrated.

Swimming

It is well documented that crayfish behaviour patterns which utilize the fast abdominal musculature are complex and varied. The giant-fibre systems, consisting of a medial and a lateral giant fibre in each connective (Wiersma, 1938), were long presumed responsible for the generation of both isolated and repeating tail flexions. Larimer *et al.* (1971) demonstrated that the behavioural outputs of the two giant-fibre systems were different, with the medials causing tight abdominal flexion and the laterals evoking flexion restricted to the rostral aspects of the abdomen. Accompanying the latter action is a flaring of the uropods. Recent analysis of crayfish escape responses in relatively free-moving animals has added to knowledge of this complex system (Wine & Krasne, 1972). Activation of medial giant fibres by rapid, anteriorly directed mechanical or visual stimuli generates a tail-flip which translates the animal directly away from the point of input. Lateral giant-fibre activity, a consequence of posteriorly directed stimuli, results in the rostral tail-flip which rotates the animal into an abdomen-up position. Subsequent action can either propel the animal over the source of the stimulus or reorient it for an alternate path of retreat. In addition to the giant-fibre systems, non-giant pathways are known to exist. Schrameck (1970) demonstrated that swimming could be initiated from either a flexed or extended position and that fast tail flexions can occur in the absence of giant-fibre activity. It was demonstrated by Wine & Krasne (1972) that in general only the initial tail-flip in a swimming sequence is triggered by one of the two giant fibres, indicating that the bulk of phasic abdominal activity is accomplished by non-giant pathways. Gradually applied mechanical stimuli, such as pinching or crushing an appendage, were successful in evoking non-giant-mediated output, as were a variety of visual inputs. It was also concluded that spontaneous swimming, which occurs in a variety of contexts, is mediated exclusively by non-giant pathways (Wine & Krasne, 1972). The non-giant systems can accomplish horizontally directed backwards movements (like MG output) or rarely upward motions (similar to LG activity). During swimming, truncated flips where neither flexion or extension are totally accomplished have also been noticed (Wine & Krasne, 1972).

Since the only known source of synaptic input to the medial giants exists in the supraoesophageal ganglion and since this has been isolated from the remainder of the system by crushing the connectives, their involvement in command-fibre-evoked tail-flips seems improbable. The lateral giants generate fast flexion of the rostral aspects of the abdomen, receiving synaptic input at all abdominal levels. Although stimulation of fibres in the circumoesophageal connective could conceivably recruit the lateral giants, the geometries evoked by most escape command elements, i.e., tight abdominal flexions, make this appear unlikely. In order to firmly verify the non-giant nature of escape responses, however, electrical activity of the giant fibres would need to be monitored during the actual performance of command fibre-evoked fast abdominal movements.

Experimenters have attempted to identify by various ablation experiments those parts of the crayfish CNS which are essential for organizing escape behaviour. Von Bethe (1897) demonstrated in *Astacus* that both single and multiple tail-flips could be generated after cutting the circumoesophageal connectives but not after more caudal cuts. These findings have been confirmed recently in *Procambarus clarkii* by Wine & Krasne (1972) utilizing mechanical stimuli which were known to activate non-giant escape pathways. Results of these ablation experiments clearly suggest that the suboesophageal ganglion is of critical import for the generation of non-giant tail-flips.

It seems probable that the various command-fibre responses presented here represent some of the non-giant pathways elucidated by Schrameck (1970), Larimer *et al.* (1971), and by Wine & Krasne (1972). It may be postulated also that the various escape command fibres isolated from the circumoesophageal connectives operate by contributing a driving excitation to neuronal oscillators located within the suboesophageal ganglion. This speculation is modelled after the swimmeret command-fibre/swimmeret oscillator systems described in the crayfish (Wiersma & Ikeda, 1964) and in the lobster (Davis, 1969*b*; Davis & Kennedy, 1972*a*, *b*, *c*).

SUMMARY

1. Command interneurons from the circumoesophageal connectives of crayfish which are capable of evoking various cyclical, phasic behavioural outputs are described. By pooling evoked-behaviour data and information on command-fibre location for a number of preparations it has been possible to repeatedly identify certain individual interneurons.

2. Five escape or swimming-command identities have been established. Electrical stimulation of any of these elements activates the fast abdominal musculature, generating single or multiple tail-flips.

3. Three forward-walking identities have been established within the experimental connective. Stimulation of a forward-walking command fibre results in extension of the abdomen and chelipeds in conjunction with locomotory movements of the walking legs. Output has been characterized as walking by virtue of the fact that the walking wheel is actually rotated by the stepping appendages.

4. Four backward-walking identities have been established. For these command elements a variety of cheliped and abdominal geometries are superimposed on the basic backward-stepping patterns of the walking legs.

5. Cyclical command fibres capable of evoking branchiostegite scratching, feeding-like activity, cleaning of the dorsal aspect of the abdomen, and slow rocking of appendages have also been noted.

The authors wish to express appreciation for the technical assistance of Gary Warinner and Ed Wake and for manuscript preparation by Mrs Kathy Nicholson. Special thanks also to Dr W. J. Davis and Mr Terry Page for comments and discussion. The work has been supported by the N.I.H.; Grant NS 05423-09 to J.L.L. and Fellowships NS 51,05401 and GM 00836-10 to R.F.B.

REFERENCES

ATWOOD, H. L. & WIERSMA, C. A. G. (1967). Command interneurons in the crayfish central nervous system. *J. exp. Biol.* **46**, 249–61.

BARNES, W. J. P., SPIRITO, C. P. & EVOY, W. H. (1972). Nervous control of walking in the crab. *Cardisoma guanhumi*. II. Role of resistance reflexes in walking. *Z. vergl. Physiol.* **76**, 16–31.

VON BETHE, A. (1897). Vergleichende untersuchungen über die functionen des central nervensystems der arthropoden. *Pflügers Arch. ges. Physiol.* **68**, 449–545.

BOWERMAN, R. F. & LARIMER, J. L. (1973). Command fibres in the circumoesophageal connectives of crayfish. I. Tonic fibres. *J. exp. Biol.* **60**, 95–117.

DAVIS, W. J. (1969*a*). Reflex organization in the swimmeret system of the lobster. I. Intrasegmental reflexes. *J. exp. Biol.* **51**, 547–63.

DAVIS, W. J. (1969*b*). The neural control of swimmeret beating in the lobster. *J. exp. Biol.* **50**, 99–117.

DAVIS, W. J. & KENNEDY, D. (1972*a*). Command interneurons controlling swimmeret movements in the lobster. I. Types of effects on motorneurons. *J. Neurophysiol.* **35**, 1–12.

DAVIS, W. J. & KENNEDY, D. (1972*b*). Command interneurons controlling swimmeret movements in the lobster. II. Interaction of effects on motorneurons. *J. Neurophysiol.* **35**, 13–19.

DAVIS, W. J. & KENNEDY, D. (1972*c*). Command interneurons controlling swimmeret movements in the lobster. III. Temporal relationships among bursts in different motorneurons. *J. Neurophysiol.* **35**, 20–9.

EVARTS, E. V. (1967). Representation of movements and muscles by pyramidal tract neurons of the precentral motor cortex. In *Neurophysiological Basis of Normal and Abnormal Motor Activities*. (ed. M. D. Yahr and D. P. Purpura), pp. 215–52. Hewlett, N.Y.: Raven Press.

EVOY, W. H. & KENNEDY, D. (1967). The central nervous organization underlying control of antagonistic muscles in the crayfish. 1. Types of command fibers. *J. exp. Zool.* **165**, 223–38.

EVOY, W. H. & COHEN, M. J. (1969). Sensory and motor interaction in the locomotor reflexes of crabs. *J. exp. Biol.* **51**, 151–69.

KENNEDY, D., EVOY, W. H., DANE, B. & HANAWALT, J. T. (1967). The central nervous organization underlying control of antagonistic muscles in the crayfish. II. Coding of position by command fibers. *J. exp. Zool.* **165**, 239–48.

LAND, M. F. (1972). Stepping movements made by jumping spiders during turns mediated by the lateral eyes. *J. exp. Biol.* **57**, 15–40.

LARIMER, J. L. & EGGLESTON, A. C. (1971). Motor programs for abdominal positioning in crayfish. *Z. vergl. Physiol.* **74**, 388–402.

LARIMER, J. L., EGGLESTON, A. C., MASUKAWA, L. M. & KENNEDY, D. (1971). The different connexions and motor outputs of lateral and medial giant fibres in the crayfish. *J. exp. Biol.* **54**, 391–402.

PAUL, D. H. (1971). Swimming behavior of the sand crab, *Emerita analoga* (Crustacea, Anomura). III. Neuronal organization of uropod beating. *Z. vergl. Physiol.* **75**, 286–302.

PEARSON, K. G. (1972). Central programming and reflex control of walking in the cockroach. *J. exp. Biol.* **56**, 173–93.

RUNION, H. I. & USHERWOOD, P. N. R. (1968). Tarsal receptors and leg reflexes in the locust. *J. exp. Biol.* **49**, 421–36.

SCHRAMECK, J. E. (1970). Crayfish, swimming: alternating motor output and giant fiber activity. *Science, N.Y.* **169**, 698–700.

USHERWOOD, P. N. R., RUNION, H. I. & CAMPBELL, J. I. (1968). Structure and physiology of a chordotonal organ in the locust leg. *J. exp. Biol.* **48**, 305–23.

VAN HARREVELD, A. (1936). A physiological solution for freshwater crustaceans. *Proc. Soc. exp. Biol. Med.* **34**, 428–32.

WIERSMA, C. A. G. (1938). Function of the giant fibers of the central nervous system of the crayfish. *Proc. Soc. exp. Biol. Med.* **38**, 661–62.

WIERSMA, C. A. G. (1958). On the functional connections of single units in the central nervous system of the crayfish, *Procambarus clarkii* (Girard). *J. comp. neurol.* **110**, 421–71.

WIERSMA, C. A. G. & MILL, P. J. (1965). 'Descending' neuronal units in the commissure of the crayfish central nervous system; and their integration of visual, tactile and proprioceptive stimuli. *J. comp. Neurol.* **125**, 67–94.

WILSON, D. M. (1961). The central nervous control of flight in a locust. *J. exp. Biol.* **38**, 471–90.

WINE, J. J. & KRASNE, F. B. (1972). The organization of escape behaviour in the crayfish. *J. exp. Biol.* **56**, 1–18.