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THE ORGANIZATION OF ESCAPE BEHAVIOUR IN THE CRAYFISH

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Invertebrate escape reflexes have long been favoured preparations for investigating neuronal substrates of behaviour. The large size of many of their component neurones make them accessible to current micro-electrode techniques, and selection pressures for rapidity of response have probably caused them to evolve in the direction of the maximal neurological simplicity consistent with their function. Indeed, given our current technical and conceptual limitations, they are probably among the very few behaviour patterns that we may presently hope to analyse with any degree of completeness.

Perhaps the most thoroughly studied of these reflexes is the escape response of the crayfish. It was first analysed electrophysiologically by Wiersma (1947) and has since been a frequent object of anatomical and physiological investigation. Wiersma, having examined the functioning of the medial and lateral giant fibres of the nerve cord, concluded (1) that either set of units calls out a similar behavioural pattern of tail flexion and streamlining and (2) that rostrally placed stimuli including visual ones excite the medial giants, while caudal stimuli excite the laterals. The presumption was that all tail-flip escape behaviour is mediated by one of these pairs of command fibres.

Recently, however, Larimer *et al.* (1971) have reinvestigated the motor consequences of stimulating each of these command fibres and found important differences between the actions attributable to each. Furthermore, Schrameck (1970) has shown that it is possible to elicit tail-flip behaviour which is not mediated by *either* set of fibres. Thus, the whole matter of the neural organization of escape behaviour in the crayfish is reopened. The purpose of the present work was to determine under what circumstances the various modes of escape are normally employed and what the adaptive significance of parallel escape systems might be.

METHODS

Procambarus clarkii (obtained from Brescia's Frog Farm; P. O. Box 3025, Compton, California 90204) of both sexes and 6–8 cm in length from rostrum to uropods were used throughout. Prior to isolation for special procedures they were maintained in groups of a dozen in 10 gal aquaria filled with dechlorinated, well-aerated water. They were fed on small amounts of liver, irregularly.

Implantation of chronic recording electrodes, as well as all other operations, were carried out after cooling an animal gradually to near 0 °C and then submerging it in ice-cold crayfish Ringer (van Harreveld, 1936). Pairs of 0·5 mm silver ball electrodes were implanted (usually in the second abdominal segment) so that they lay near to the

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abdominal cord and in favourable cases rested against its dorsal surface immediately adjacent to the giant fibres. The leads were threaded through the animal and out of a hole in the opposite tergum, where they were secured with Eastman 910 adhesive. In almost all cases potentials due to giant-fibre activity could easily be seen. In some animals a second pair of electrodes was implanted near a circumoesophageal connective either through an opening in the epistoma or by inserting pin-type electrodes through the carapace (Aréchiga & Wiersma, 1969). Implanted animals were maintained in separate 5 gal aquaria with floats attached to their electrode leads. They could be kept indefinitely.

In several animals after completion of behavioural study the cord giants were directly stimulated to confirm the identification of giant-fibre potentials. Animals were pinned ventral side up in crayfish Ringer, a flap over the last abdominal ganglion and connective was opened, the roots from the last ganglion were severed, and the cord was folded forward to expose its dorsal surface. An insulated stainless-steel electrode with a fine, bare tip was placed directly over the desired giant fibre and stimulated at threshold intensity. Responses were recorded via the same indwelling electrodes that had been used in the freely behaving animal.

The tail-flip responses of a number of animals (both unoperated and implanted) were filmed with a Wollensack Fastaire camera operated at 100 or 160 frames/sec, and electrical records of the implanted animals were later correlated with response patterns.

Response latencies to tactile stimuli were obtained, where possible, by utilizing the output of a microphone attached to the metal stimulating rod as a stimulus marker.

Nerve and muscle potentials were amplified by a Tektronix 122 preamplifier and recorded on AM tape. The limited dynamic range and poor low-frequency response of this recording method sometimes resulted in clipping or distortion of muscle potentials, but nerve spikes were recorded accurately. Oscillographic display and photography were conventional.

In a final series of experiments the ventral cord was transected at various levels. Access to the abdominal cord was gained by slitting the ventral integument; the thoracic cord was exposed by splitting the fused sterna along the midline and retracting them; the circumoesophageal connectives were reached through an opening made in the epistoma. In all operations nearby arteries were spared.

RESULTS

Anatomical review

Four giant axons dominate the dorsal surface of the ventral nerve cord (Johnson, 1924). The medial pair (MG's) originate from cell bodies in the brain, decussate, and travel without interruption to the last abdominal ganglion. In the brain they send branches towards incoming fibres from the antennae and antennules from which they presumably receive sensory input directly or via internuncials (Horiuchi, Hayashi & Takahashi, 1966). They also appear to synapse with one another as they decussate, and an impulse in one often fires the other (Wiersma, 1947). The MG's reach their greatest diameter, up to 200 μm, in the circumoesophageal connectives and taper to about 150 μm or less in the abdomen (Fig. 1).

The lateral pair of giant axons (LG's) are actually composed of partially fused segments (Payton, Bennett & Pappas, 1969), each of which originates from a heterolateral ventral cell body in each ganglion (Remler, Selverston & Kennedy, 1968). The neurites which connect the dorsal axonal segments to the ventral somas extend dendritic branches and receive input along much of their length (Selverston & Kennedy, 1969; Krasne & Stirling, 1971), and where they cross the midline they synapse electrically with their contralateral homologues so that an impulse initiated in one LG necessarily fires the other (Kusano & Grundfest, 1965). The LG's are largest in the abdomen, up to 200 μm, and taper to about 80 μm in the circumoesophageal connectives (Fig. 1).

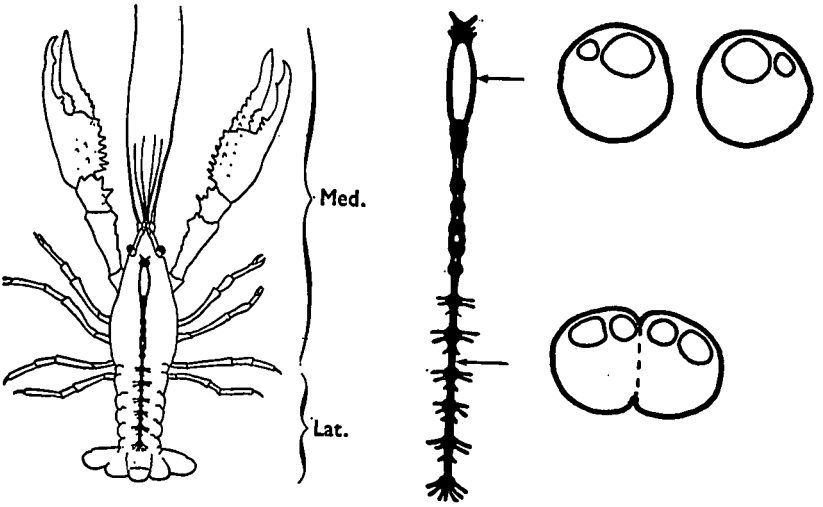

Fig. 1. Receptive fields and axon diameters of the giant fibres of the crayfish. Approximate receptive fields for MG's (Med) and LG's (Lat) are shown on the left. On the right are cross-sections of the nerve cord taken at the levels indicated on the centre diagram. Notice the relative sizes of the giants at each level (sections after Wiersma & Hughes, 1961, and Wiersma & Mill, 1965).

Both pairs of giant interneurones make a complex set of efferent connexions which are responsible for the co-ordinated patterns of muscle contractions that produce tail flips and the various adjustments of appendages which accompany them. Some of these connexions are known to be direct motor neurone contacts (Takeda & Kennedy, 1964; Remler *et al.* 1968); others have not yet been investigated.

Identification of cord giant activity

The muscle potentials associated with tail-flip responses were often preceded by large-amplitude spikes in the ventral cord. Since these occurred only in association with tail-flip behaviour (about 2 msec before muscle potentials of the abdominal flexors) and in most preparations were 5–10 times larger than any other spikes seen, it is likely that they represented activity of the cord giants. This was confirmed by the results described below.

Two criteria were used to differentiate LG's from MG's. (1) Bipolar electrodes situated rostrally in the abdomen record polarity differences between ascending and

descending activity (Fig. 3); it was assumed that ascending spikes, which had originated in the abdomen, must be in LG's, since MG's show no sign whatever of receiving synaptic input in the abdomen (Kao, 1960; Krasne, unpublished observations), and conversely, descending activity was assumed to be in the MG's. Support for this latter assumption came from observations during simultaneous recording from circumoesophageal connectives and abdominal cord (see below), where descending giant spikes could readily be identified on the basis of spike size as being in the MG's. (2) The flexion patterns evoked by LG's and MG's (Larimer *et al.* 1971; see also Fig. 5) produce different trajectories of escape. The LG's evoke a forward pitch and a lift while the MG's cause a dart backwards.

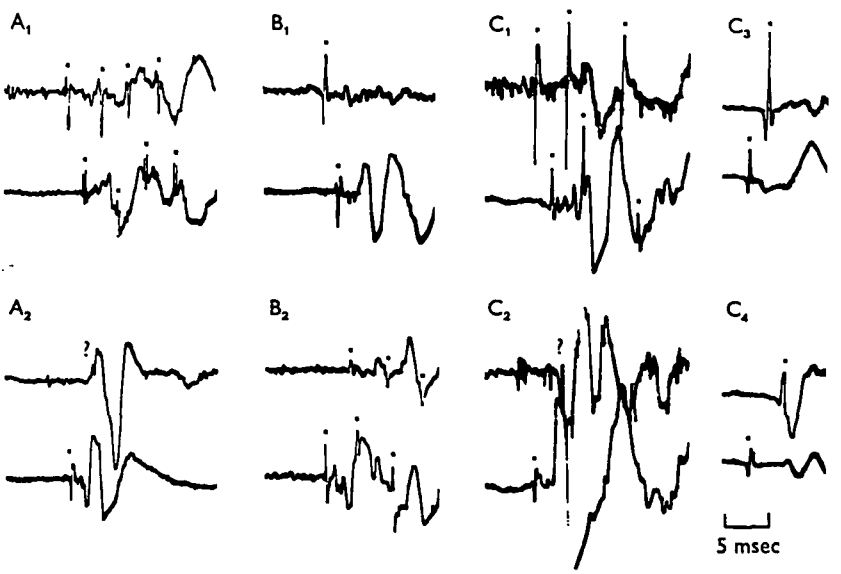

Fig. 2. Giant-fibre responses in doubly implanted animals. The upper pair of traces shows MG responses and the lower pair LG responses, throughout. The upper trace of each pair is from circumoesophageal connective leads and the lower from electrodes in the second abdominal segment. A, B and C are from different animals. Records 1 and 2 from each animal are responses to natural stimulation. Giant-fibre spikes are marked by dots or in uncertain cases by question marks; slower voltage swings in this and other records are muscle potentials. Note that in each animal reflexly evoked MG spikes are large in the connective, descending, and opposite in polarity to LG spikes at abdominal leads, while the converse is true for LG spikes. C_3 and C_4 are responses to direct stimulation of the giants under visual control (see methods). Differences in size and form between the directly elicited and reflexly evoked spikes are due to greater synchrony of the LG's when reflexly evoked and to failure of the directly elicited MG spikes to cross the brain's commissural synapse in this particular experiment.

Although neither of these differences formed an absolutely reliable criterion for identification, used together they provided a convincing basis for discrimination in about 90% of the cases examined. Our conclusions for this 90% of unambiguous responses were further validated on nine animals with an extra set of recording electrodes implanted near the circumoesophageal connectives. The MG's are more than twice the diameter of LG's at this level (Fig. 1), and hence the fibres can be differentiated readily on the basis of spike size. Latency differences between the electrodes also permit unequivocal determination of direction of propagation. Spikes

thought to be from MG's by all other criteria were in fact always several times larger in the circumoesophageal connectives than spikes thought to be from LG's (Fig. 2).

Finally, the giants of five of these double-electrode animals were directly and selectively stimulated under visual control (see methods) at the termination of behavioural experiments, and previous identifications were again confirmed (Fig. 2).

We are sure enough of our identifications to be confident of the generalizations set forward below. However, given that some 10% of our cases were ambiguous, we cannot dismiss the possibility that occasional exceptions to these generalities may occur.

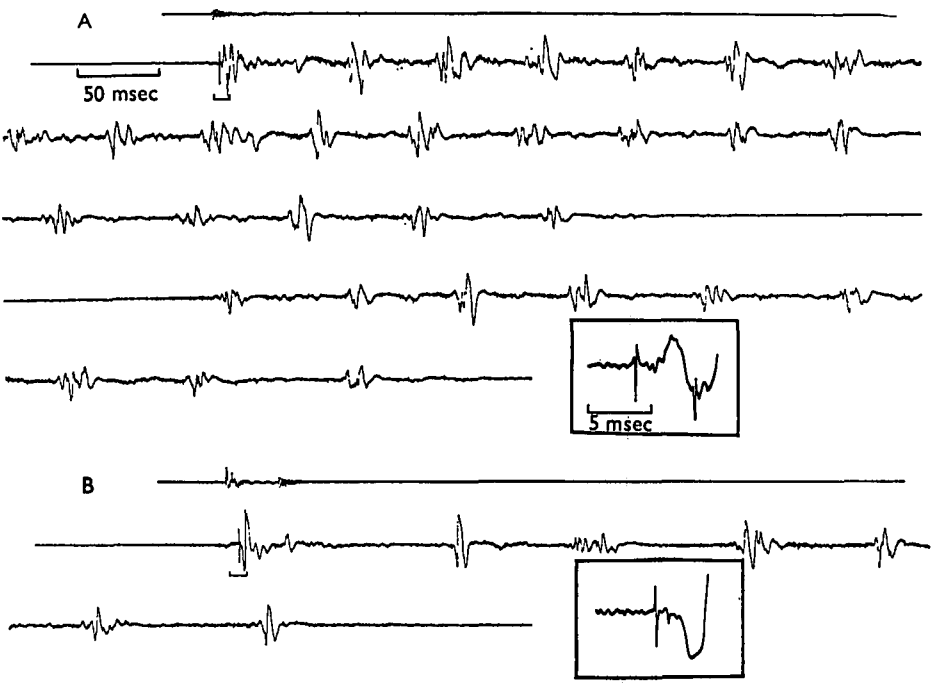

Fig. 3. Giant-fibre responses followed by swimming. Successive traces are contiguous segments from moving film records of abdominal cord output; the top line in each series is the stimulus-transducer output used to measure response latencies. Insets show details of the initial (bracketed) portion of each series. A, A tap to the rostrum elicits an MG-mediated flip (in this case two MG spikes were initiated) followed by non-giant swimming. Notice that the initial flip in the second swimming series, which occurs after a 350 msec pause, is also a non-giant response. B, A tap to the abdomen elicits an LG-mediated flip followed by non-giant swimming. The second defection on the transducer trace was caused by the animal's abdomen striking the stimulator during flexion.

The organization of escape behaviour

Differentiation of giant and non-giant systems

Extensive exploratory experiments on restrained animals showed that giant-fibre responses can be elicited by brief shocks to first or second abdominal roots or by sharp taps to the side of the abdomen but not usually by gradual pinching, even to the point of breaking sternal ribs, by prodding, even to the point of piercing the ventral cuticle, by making extensive incisions of the belly, or by mashing, cutting, or pulling off appendages. However, these latter kinds of stimulation often do evoke tail-flip

responses which are not mediated by giant fibres. While such reactions sometimes appear to be elicited directly by the stimulus, they often seem instead to be a part of the general struggling behaviour which such stimuli may induce. Atwood & Wiersma (1967) have shown that several command fibres of the circumoesophageal connectives when stimulated at frequencies well in excess of those required to produce a variety of specific reactions also evoke general struggling and tail flips; however, whether the giant fibres are silent in these cases has not been reported. Non-giant flips also occur, and not infrequently, without immediate provocation (see below).

The differentiation of systems is illustrated by the following experiment. Five crayfish were fixed to corks above their thoracic carapaces; thus immobilized they were submerged dorsal side up with their abdomens held extended by a loop of thread. A given abdominal segment was then stimulated every 5 min, either by tapping or by squeezing, the two types of stimulation being varied randomly. The squeeze was

Table 1. *Differentiation of two escape systems by measuring differential responsiveness to two kinds of stimulation in restrained crayfish*

Stimulus ...	Pinch	Tap
No. of trials	94	52
Trials with responses (%)	54%	58%
Responses with giant fibre spikes (%)	10%	97%

administered with a pair of forceps with attached springy wires that gave way when the forceps were closed. Closure was carried out so that about 0·5–1 sec elapsed between contact and full pressure, which was then maintained until the animal had responded or 3 sec had elapsed. The strength of the tap stimulus was adjusted so that the probability of a response was about the same as when a squeeze stimulus was employed. Eight to 15 stimuli were given during sessions which were separated from one another by 24 h. Although the two stimuli were about equally effective in producing tail flips, there was a striking difference in the way the flips were mediated (Table 1). Responses to the tap stimulus were almost always mediated by the giant fibres while responses to the squeeze stimulus were rarely associated with giant-fibre activity. We think it likely that the lack of perfect separation was due primarily to overlap in stimulus characteristics, for one would expect a sensitive animal to respond to the initial contact of a squeeze stimulus as though it were a tap.

Escape behaviour of freely moving crayfish

Although stimuli can be best controlled and recording optimized in restrained animals, it is essential to examine escape behaviour of relatively free animals, because the excitability of the various escape systems is subject to inhibitory influences and can be markedly altered by restraint (Wine & Krasne, 1969, and in preparation).

Chronically implanted, freely moving crayfish were induced to escape by tapping and pinching various parts of their bodies, by passing shadows overhead or moving objects toward them, by attempting to pick them up, etc. These stimuli were given at our convenience at rates up to several times a minute. Testing sessions lasted from 5 to 30 min and were terminated when escape behaviour became difficult to elicit; usually 10–20 responses were obtained in a session. Not more than two such sessions

were given in any one day. Prior to or during sessions, crayfish sometimes backed into corners and curled their abdomens beneath them. When this occurred they were usually moved into the open by pulling lightly at their recording leads or by pushing them gently with a rod. Hence, stimuli were very commonly delivered to alerted animals.

It should be understood that our descriptions of stimuli are operational and do not tell what the animal perceived. Most stimuli delivered to animals under water have tactile, visual, vibratory, and possibly chemosensory components. Nevertheless, it was found that a given operationally defined stimulus usually provoked escape via a particular system. Thus it was possible to characterize the adequate stimuli for three modes of escape: MG, LG, and non-giant.* Much of the overlap in the categorization must have been due to the multidimensionality of the stimuli employed, and it seems likely that with better stimulus control the stimulus-response categories would have been even more sharply defined.

Table 2. *Stimulus–response relationships for unrestrained crayfish*

Stimulus	No. of responses obtained			No. of animals observed
	LG	MG	Non-G	
Tap abdomen	81	0	7	20
Touch antenna or tap rostrum	0	56	5	16
Tap thorax, claws or rostral legs	0	10	4	2
Very rapidly approaching object (visual)	0	10	0	2
Pinch antenna or leg	0	2	17	7
Pinch uropods	1	0	12	3
'Ordinary' visual	0	1	15	3
Non-specific: pull leads, handle, etc.	0	1	8	3
'Spontaneous'	0	0	15	5

Lateral giant system. Of the various stimuli tested on free animals (Table 2) only taps to the abdomen reliably elicited LG responses. This and a variety of observations on restrained animals have convinced us that giant-fibre activity evoked by caudal stimuli occurs only in the LG's and conversely that only caudal stimuli can fire these fibres; however, we have not attempted a precise determination of the rostral extent of the receptive field of the LG reflex.

Whereas it is generally believed that the LG reflex is a high-threshold system requiring strong or noxious stimuli for its activation (Wiersma, 1947 and 1961; Krasne & Woodsmall, 1969), we find that in free animals intensity is not as important as suddenness. Very light taps are often effective, and since the LG's occasionally fire just prior to actual contact, it may be that even the pressure waves in advance of a rapidly approaching object can be an adequate stimulus. Careful tests of threshold were not conducted, but the pleural plates seem to be more sensitive than the dorsal parts of the terga, and the caudal pleural plates more sensitive than the rostral ones.

* Although tail-flip behaviour seems most often to be used for escape from external threats, we have also seen it occur under the following conditions: (*a*) during extrication from the old exoskeleton during moulting, (*b*) following loss of balance during climbing, (*c*) as part of efforts to free bits of food held in forceps, and (*d*) to transport an animal to water plants floating at the surface of a sheer-walled aquarium. We have not determined which of the tail-flip systems are operating during these performances, but the picture which has emerged from our observations on escape leads us to believe that the giant fibres are not involved.

Although it is known that a single impulse initiated in any of the central giants can produce a tail flip (Wiersma, 1947; Roberts, 1968), the response to natural stimuli is often a brief burst of up to four spikes (Figs. 2, 3). The median latency to the first spike was 7 msec (Fig. 4), and subsequent spikes followed at frequencies of 125–500 impulses/sec. The duration of the bursts ranged from 5 to 33 msec.

The occurrence of multiple spikes is surprising because Roberts (1968) has shown that there is a recurrent inhibitory loop by which the LG's inhibit their own firing for about 90 msec following a single spike. Perhaps in the intact animal central processes can dampen this inhibitory circuit or excitation is strong enough to compete successfully with it. Certainly the duration of the compond EPSP generated in the LG's even by highly synchronous afferent volleys is easily long enough (Krasne, 1969) to account for our observed burst durations, and even in the isolated abdomen similar bursts can be produced in preparations whose inhibitory processes are poisoned with picrotoxin (Roberts, 1968).

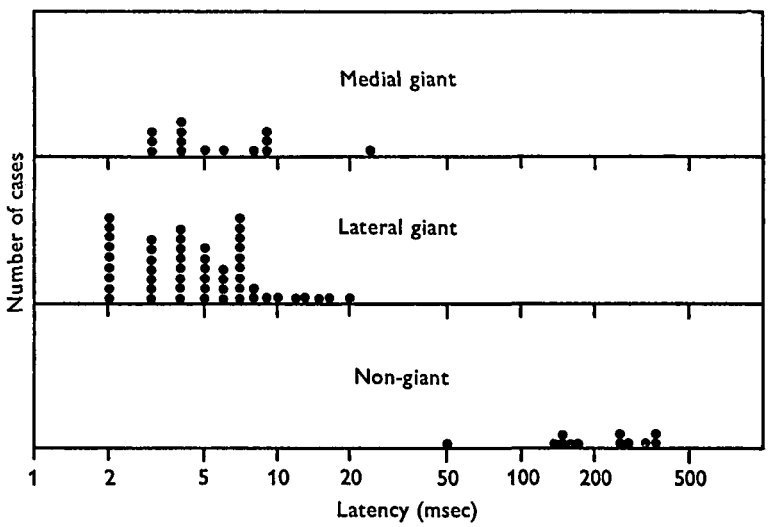

Fig. 4. Escape reaction latencies. Latencies (plotted on a log scale) were measured from the time of audible contact of the mechanical stimulator (see text) till the first giant-fibre spike or to the first major muscle potential in the case of non-giant reactions. Giant-fibre spikes invariably lead to muscle potentials in about 2 msec. No correction was made for the time (about 0·5 msec.) required for propagation of the sound pulse to the microphone.

The repetition of spikes might be expected to ensure an adequate response by facilitating and summating at LG-motoneurone junctions (Wiersma, 1947; Wiersma & Shalleck, 1947) and neuromuscular junctions (Kennedy & Takeda, 1965).

The abdominal flexion which is evoked by this LG firing causes the animal's body to pitch forward about an axis near the chelipeds and causes a lift which, especially in smaller animals, propels the animal dorsally (Fig. 5; see also Larimer *et al.* 1971). This action of a system primarily designed for backward movement answers the dilemma posed by threats coming from the rear; the movement upward sets the stage for escape trajectories directed over or away from the site of attack (see below). The streamlining of appendages which is usually considered as a characteristic of giant fibre-mediated reactions seems to be absent from responses mediated by the LG's.

Medial giant system. The MG's, like the LG's, have previously been characterized as high-threshold interneurones (at least in so far as their responses to tactile stimuli are concerned) (Wiersma, 1961). Although this is sometimes so, their excitability varies enormously. In free animals the lightest touch of an antenna or antennule may elicit MG responses, and under some circumstances these can be evoked repeatedly though under other circumstances they may habituate readily. Taps to the rostrum, legs, claws, and rostral thoracic carapace can also fire the MG's whereas more gradual stimuli, even when injurious, usually do not (Table 2).

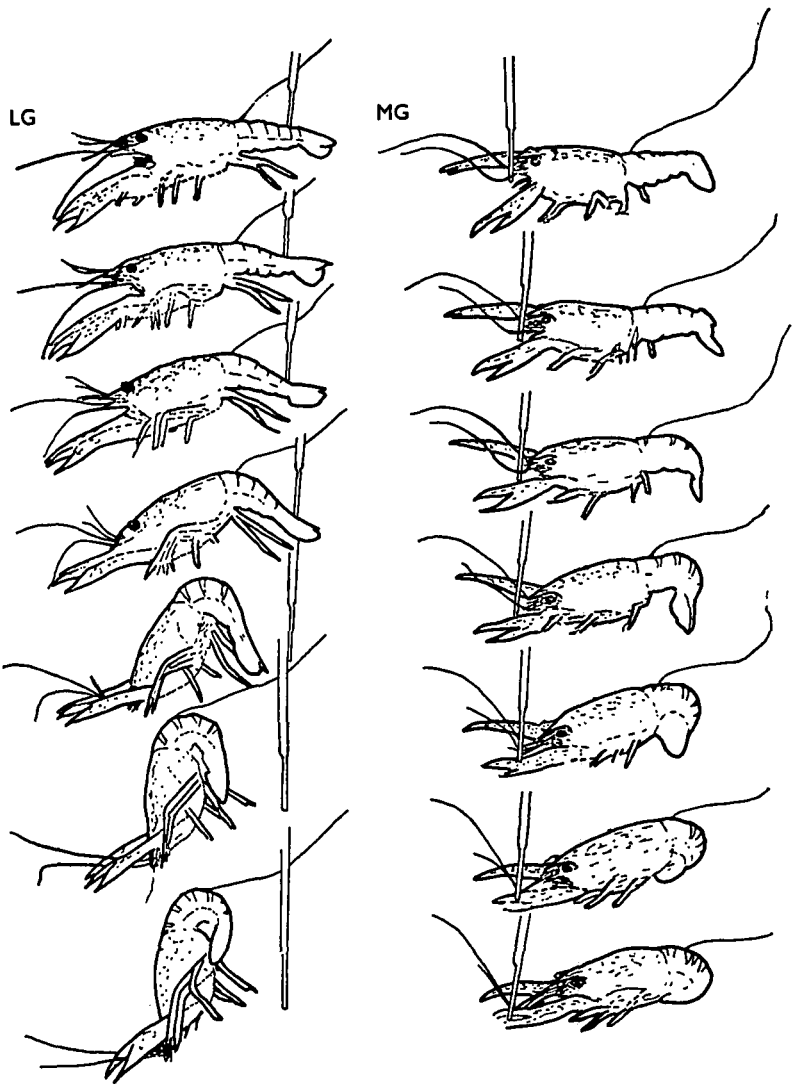

Fig. 5. Tracings from high-speed motion pictures of MG and LG reflex tail flips in unrestrained animals. The trajectories of escape, which are quite stereotyped and different in the two cases, confirm predictions based on observations in restrained animals (D. Kennedy, personal communication; Larimer *et al.* 1971). The frames selected for tracing are about 10 msec apart; the prod used to stimulate the animal and recording electrode leads are shown.

The MG's are reputed to be readily excited by visual stimuli (Wiersma, 1961), but this conclusion, which was probably based on behavioural data, was reached without cognizance of the possibility of non-giant responses. We find that although a large variety of visual stimuli including sharp-edged moving shadows, black objects approaching under water, light onset and offset, and movements of the experimenter can, in varying degrees, elicit tail-flip behaviour, they very rarely fire the MG's. It may be that similar stimuli, appropriately chosen or properly timed, could excite the MG's with some regularity, but in our hands the only visual stimulus capable of doing so has been a rapidly approaching object (e.g. an open palm or folded newspaper) coming within inches of an animal at full velocity.

In order to convince ourselves that such stimuli were not affecting the animals via air or water currents attendant upon their approach, several animals were placed in shallow containers and covered by a sheet of glass which rested on a separate, cushioned support about 1 in. above the barely submerged crayfish. The glass was then slapped with a lightly folded newspaper once every 5 min; on alternate trials the glass was covered with opaque paper to control for slight vibrations which were transmitted to the animal via the table top and could when strong enough excite the giant fibres. Although some animals did not respond at all and the remainder habituated rapidly to such stimuli, about a dozen responses were collected. Since none of these occurred on trials when the glass was covered, we believe that they really were reactions to the visual stimulus.

Precise response latencies of the MG's were difficult to determine. Our microphone transducer was not sensitive enough to detect the touching of an antenna, which was the most effective stimulus found, and taps to hard parts of the head region, which the transducer could sense, probably excited visual and antennal receptors before audible contact was made. Nevertheless, latencies of MG responses to tapping the rostrum and to sudden visual stimuli (timed from the moment of contacting the glass which covered the animal) can be seen (Fig. 4) to be in the same general range as those of LG responses to caudal stimuli; the median latency was 4 msec.

The MG's often responded with a brief burst of spikes – even to a visual stimulus or to a light touch on the antenna. Trains of up to 7 spikes have been seen (Figs. 2, 3).

When the MG's fire, the abdomen and uropods are flexed in such a way that the animal darts backward along a low trajectory and at the same time the claws and legs flick forward to streamline the animal (Fig. 5; see also Larimer *et al.* 1971).

Non-giant responses. Whereas in restrained animals non-giant-mediated tail flips occur mainly as responses to intense or traumatic stimulation or as part of spontaneous struggling movements, in free crayfish a variety of subtle stimuli can bring about similar responses. Attempts to move animals by gently pushing them, by pulling lightly at their recording leads, or by very gently picking them up often led to tail flips. They also reacted when shadows passed overhead, when someone approached their aquaria, when there were other movements in the room, or, sometimes, in the absence of any apparent stimulus at all (Table 2). Limited testing with pinches to uropods, abdomens, antennae, antennules, and legs also confirmed that free crayfish, like restrained ones, usually respond to gradually increasing stimuli without activation of the giants (Table 2).

Although it is not feasible to determine accurate response latencies in situations

like the above, occasionally a sudden stimulus of the sort which usually fires the giants will evoke a non-giant flip instead. In these cases the latencies which were obtained were always at least an order of magnitude greater than those of giant-fibre responses to comparable stimuli (Fig. 4).

Because non-giant flips of the sort under discussion tend to occur at relatively unpredictable moments, none have been filmed; however, it is clear from direct observation (see also the following section on swimming) that they may propel animals either backward (as do MG mediated flips) or, more rarely, upward (as do LG mediated flips).

Swimming. Free, rested crayfish most often escape not by a single tail flip but by a sequence of flips (*swimming*). The results described above apply to single responses and to the initial responses of swimming sequences. Subsequent flexions are virtually never mediated by giant fibres; of over 1000 flexions contained in about 100 swimming sequences by 13 animals, giant-fibre activity occurred after the first flip on only five occasions, and in each case there was reason to suspect that the giants might have been triggered by stimuli such as the animal bumping into a wall of the aquarium or breaking the water surface (see Discussion).

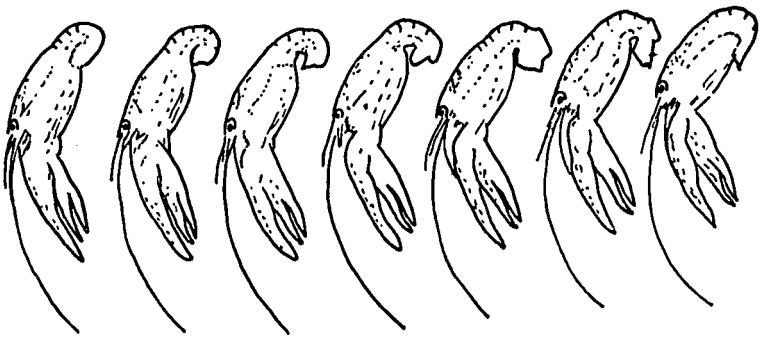

Fig. 6. Truncated tail flip from a swimming sequence (see text). This is one example of the flexibility of tail flips during swimming. Time between tracings, about 10 msec.

Swimming behaviour subsequent to the initial flip of a sequence is complex. A variety of flexion patterns such as truncated flips (Fig. 6) in which neither flexion nor re-extension are completed or flips similar in form to those produced by the MG's or LG's are all common. The timing and sequence of these patterns is also flexible with an initial LG-mediated flip, for example, being followed either by propulsion directly backward (from the animal's point of view), which results in a high, arcing trajectory, or by continuing LG-type (but not LG-mediated) flexions, which often cause a complete somersault that is in turn followed by a roll and backward swimming (Fig. 7).

Preliminary analysis of the non-giant system(s)

We have attempted to get some feeling for the anatomical organization of the non-giant mediational systems by examining the effects of cuts at various levels of the nerve cord. Von Bethe (1897) long ago reported that in *Astacus* both single tail flips

and on occasion swimming could be elicited after cutting circumoesophageal connectives (see also Schrameck, 1970) but not (or at least very rarely) after more caudal cuts. This suggested that the suboesophageal ganglion might be critically involved in non-giant responses. The results reported below confirm this.

Each operated animal was tested about once a week for 3 months following surgery. Single tail flips and swimming in response to gradual caudal stimuli of the sort which

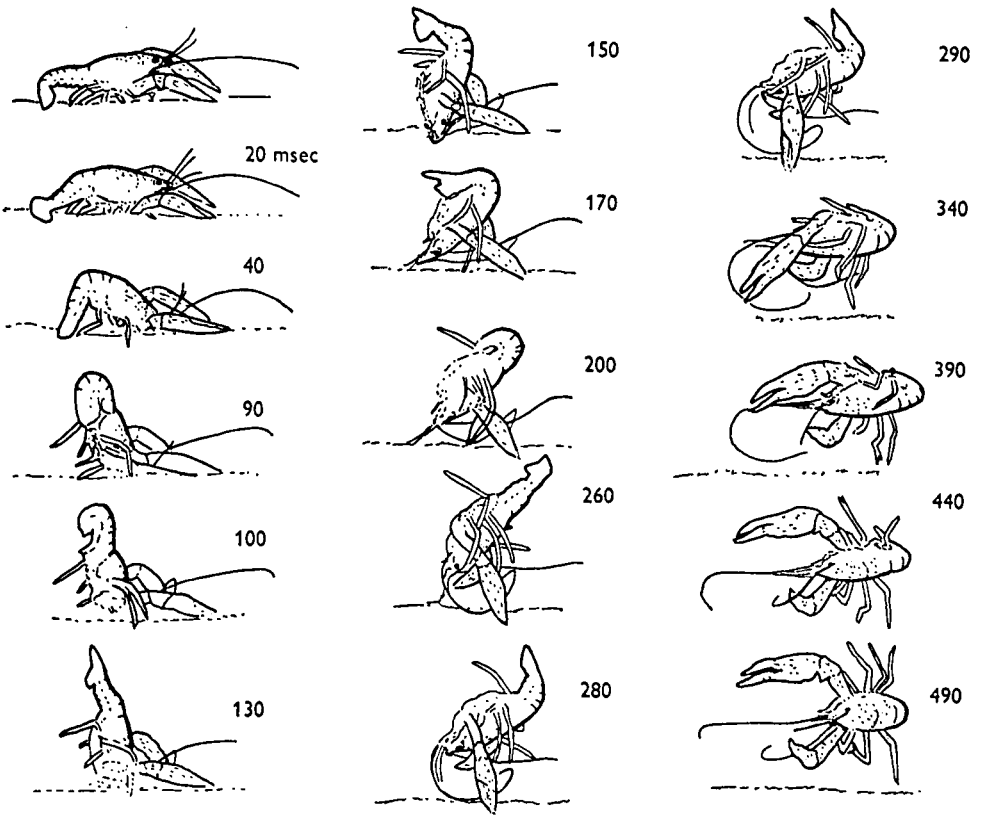

Fig. 7. A somersault response. A tap to the animal's abdomen caused an LG response followed by several non-giant flips of LG type and then by a sequence of truncated flips (only partly shown) which rolled the animal to an upright position a short distance from, and facing, the stimulator; the sequence terminated with the animal in a defence posture (not shown). Note lack of streamlining.

normally evoke non-giant responses could be routinely elicited after cutting circumoesophageal connectives but not after cutting just behind the suboesophageal ganglion complex (Fig. 8, Table 3). After more caudal thoracic cuts (Fig. 8, Table 3) gradual pinching of antennae could elicit what appeared to be rostral components of swimming such as forward thrusts and intermittent twitches of appendages, but non-sudden stimuli caudal to the cuts were now ineffective. From this it might be concluded that the suboesophageal ganglion complex originates commands for non-giant tail flips. Experiments with hemisections of the cord are consistent with this interpretation (Fig. 8, Table 4). To casual observation non-giant tail flips and swimming seem normal in hemisected animals. However, systematic testing showed that animals with

hemisections of the circumoesophageal connectives would not respond even to very strong pinches of the ipsilateral antenna despite the fact that MG-mediated flips remained bilaterally elicitable by light taps and that the threshold for responses to contralateral pinches was approximately normal. After more caudal hemisections responsiveness to rostral stimuli was normal, but the threshold for pinch stimuli delivered behind and ipsilateral to the cut seemed to be elevated. These results may be interpreted to mean that the sensory input reponsible for non-giant responses is conveyed

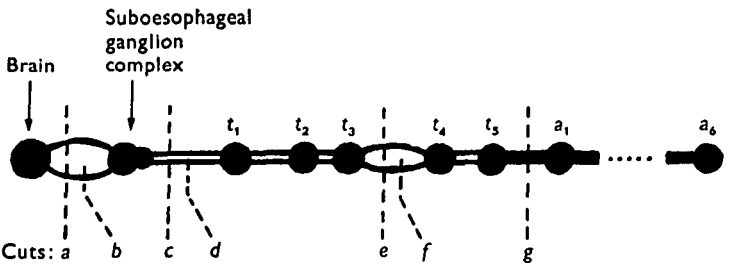

Fig. 8. Locations of cuts whose effects are shown in Tables 3 and 4. Thoracic ganglia are denoted by t_1–t_5 and abdominal ganglia by a_1–a_6. The cord is not drawn to scale.

Table 3. *Effects of isolating various portions of the CNS on giant and non-giant responsiveness*

	Level of cut			
	Circum-oesophageal connectives (Fig. 8; *a*)	High thoracic (Fig. 8; *c*)	Low thoracic (Fig. 8; *e*)	Abdominal-thoracic connective (Fig. 8; *g*)
No. of animals	5	3	3	10
Responds to tapping side of abdomen	Yes	Yes	Yes	Yes
Responds to pinching uropods	Yes	No	No	Developed gradually
Swims	Yes	No	No	No

Table 4. *Effects of hemisections at various levels on giant and non-giant responsiveness*

	Operation		
	Cut one circum-oesophageal connective (Fig. 8; *b*)	High thoracic hemisection (Fig. 8; *d*)	Low thoracic hemisection (Fig. 8; *f*)
No. of animals	2	2	2
Responds to tapping either side any level	Yes	Yes	Yes
Swims	Yes	Yes	Yes
Responds to pinching contralateral to cut; any level	Yes	Yes	Yes
Responds to pinching ipsilateral antenna	No	Yes	Yes
Responds to pinching ipsilateral legs	Yes	Decreased	—
Responds to pinching ipsilateral uropods	Yes	Decreased	Decreased

to the suboesophageal ganglia over largely uncrossed pathways and that commands for non-giant escape are issued bilaterally or can cross readily from one side of the cord to the other.

While the above picture is a consistent one, other evidence indicates that non-giant responses can originate segmentally. Electro-physiological experiments with isolated nerve cords reveal that sensory input can directly excite flexor motor neurones which participate in tail-flip behaviour (Takeda & Kennedy, 1964). Furthermore, about a week after bilateral interruption of the thoracic-abdominal connectives, which initially abolishes non-giant responses to caudal stimuli, such responsiveness begins to return. Why recovery should occur in this particular preparation is not clear (the phenomenon will be described further elsewhere: Wine, in preparation), but it does seem to render likely the possibility of segmental initiation of non-giant flips. On the other hand swimming by repetitive flexions never returns in these animals, and animals cut at higher levels (but still below the suboesophageal ganglion complex) do not show segmentally initiated non-giant flips. Taken as a whole, the evidence points to an important role for the suboesophageal ganglion complex in the mediation of non-giant tail-flip behaviour, either as initiator, or facilitator, or both.

DISCUSSION

The organization of escape behaviour

A number of the observations detailed in the preceding pages are at variance with previously accepted views about the crayfish giant-fibre reflexes. We find that the giants are not primarily high-threshold systems responding mainly to intense or noxious stimuli, that they are not involved in many naturally occurring escape reactions and in particular not in 'spontaneous' reactions, that they commonly fire in bursts, and that they initiate responses but are not involved in swimming. While these results might have arisen from any number of procedural idiosyncrasies, we attribute them largely to our use of intact and unrestrained animals in contrast to the variously reduced preparations that have been utilized in previous work.

In any case they provide a consistent and plausible picture of the organization of tail-flip behaviour in the crayfish. Commands for response can be issued in at least three ways. The mode utilized is determined by the locus and nature of the stimulus and has important consequences for various features of the resultant response.

Sudden stimuli tend to fire the giant fibres. The decision to fire is itself rapid – central delay is often only a few msec – and immediate effector consequences are assured by a system of large axons and minimal synaptic delays. The cost of such speed and reliability is high; the responses are quite stereotyped, and essential flexibility requires two sets of giant fibres – the MG's for escape from anterior stimuli and the LG's for escape from posterior stimuli.

Less sudden stimuli, which presumably are typically associated with less precipitous threats, do not activate the giant fibres but are instead dealt with by the slower but more flexible non-giant system(s). In contrast to the giant-fibre reactions, which are properly considered as reflexive, non-giant reactions seem to be essentially voluntary in nature. Animals very much appear to choose their own moments to respond; latencies are at best an order of magnitude greater than those of giant-fibre reactions;

and often it seems more that a stimulus has set the occasion for a non-giant response than that it has strictly elicited it. In fact, these responses are frequently entirely spontaneous in the sense that they may occur in the absence of any environmental change of a kind which would normally be adequate to evoke escape, though we may perceive various possible disturbances of long standing that could have been 'motivating' factors. We believe, but have not established, that the longer latency of these reactions provides time for the animal to adjust his response to the situation. Both giant-mediated and non-giant-mediated tail flips can be followed by sustained swimming, and this is mediated almost exclusively by non-giant systems. We do not know the functional significance of the giant-fibre activity occasionally observed during swimming (see Schrameck, 1970, for a discussion), but it appears to us that the primary function of the giant fibres is to initiate escape from precipitous treats.

Non-giant reactions

Giant-fibre escape reactions are widespread among higher invertebrates, and it is commonly assumed that where giant interneurones are found they are essential for the response patterns which they are known to evoke. However, it has been known for some time that the rapid withdrawal response of the tube worm *Branchiomma vesiculosum* can be mediated by a non-giant system once the giant-fibre reflex has become habituated (Krasne, 1965), and it has recently been shown (Dagan & Parnas, 1970) that the evasion response of the cockroach likewise does not depend essentially on the giant interneurones usually supposed to mediate it. These systems have not been fully explored, and we do not know to what extent they are analogous in function to the non-giant systems of the crayfish. However, it does now seem likely that non-giant systems will commonly be found to parallel giant ones.

Our observations have provided few details about non-giant escape, but the range of movements seen during swimming argues for a control system of relatively complex design. It seems likely that the organization of this system will prove to be generally analogous to that of the command systems controlling abdominal posture (Kennedy & Takeda, 1965; Evoy & Kennedy, 1967; Kennedy *et al.* 1967), slow uropod movements (Larimer & Kennedy, 1969) and swimmeret movements (Ikeda & Wiersma, 1964; Wiersma & Ikeda, 1964; Davis, 1969*a*, *b*; 1970). In all these systems a given motor neurone may potentially be driven by a large number of command interneurones (about 40 for the swimmeret system) each of which also influences hundreds of other efferent elements in a number of segments in a manner which determines a unique pattern of movement.

Decision processes

Each time a crayfish is exposed to a potentially threatening stimulus the animal's nervous system must decide whether to act and must select a particular response from the limited repertoire which comprises escape behaviour. This task may be thought of as a model in miniature of that performed in elaborating behaviour as a whole, and for this reason the mechanisms of its execution are of the greatest general interest.

That escape provides a plausible general model of behaviour must admittedly be questioned on the grounds that evolution for speed may have made it highly special. Thus, ethologists believe behaviour to be organized hierarchically, yet escape cannot be so because there is not time first to decide to take evasive action and then to choose

a manner of doing so. The decision of whether or not to act is in fact made largely independently for each mode of escape on the basis of the characteristics of the applied stimulus; this is a situation which obviously poses problems of co-ordination (see below) and may well be exceptional.

To this possible drawback of using the escape repertoire as a model system, however, must be opposed its great susceptibility to neurophysiological analysis. The LG reflex, in particular, is among the very few behaviour patterns known for which representative links of the entire neuronal pathway between stimulus and response have been elucidated, and its inclusion as part of a functionally understood system of related behaviours makes it at present a unique object for analysis. The LG reflex pathway is composed of receptors, a single-tiered network of parallel, mutually interacting interneurones (Zucker, Kennedy & Selverston, 1971), a command neurone with an essentially binary output code, and a well-elucidated motor apparatus; by analogy the MG pathway is probably organized similarly. This scheme thus provides a framework which permits us to ask detailed questions about the organization of the central processes which underlie decision and choice.

How is the stimulus filtering which is responsible for the selective responsiveness of the LG's to sudden stimuli achieved? The interneurones which feed the LG habituate rapidly to maintained or repeated sensory input (Zucker *et al.* 1971; Krasne, 1969); does this account for the LG's stimulus selectivity? Do these rapidly habituating sensory interneurones comprise private recognition circuitry for the LG's, or are they members of a common pool of sensory interneurones which abstract different features of the ganglion's sensory input and feed all those command neurones to which a given feature is relevant?

What are the mechanisms whereby one act is performed at the expense of others? It is already known that firing of the MG's produces in the LG's an IPSP which attenuates LG input for the duration of a tail flip (Roberts, 1968) and that during swimming behaviour (at certain phases of each cycle) transmission between LG's and motor neurones, which is normally obligatory, is blocked (Schrameck, 1970). Might it be a general rule that commands for one behavioural act directly inhibit the commands for potentially competing acts? Do checks operate at other levels? Can activity of the assemblage of interneurones which feeds the LG alter the excitabilities of acts synergistic or antagonistic to LG escape before the LG itself actually fires? Conversely, can the likelihood of LG escape be influenced not only by facilitation or inhibition of the LG itself but also by regulation of the activity of earlier interneurones, thereby biasing the developing 'intention' to respond?

It seems to us that questions such as these will be central to the analysis of decision processes wherever they are studied. For the crayfish escape repertoire, however, they can be answered with immediately available techniques, and while it cannot be maintained that the answers will have *direct* general applicability, we do not consider it naive to suppose that they will have important implications for our understanding of behaviour as a whole.

SUMMARY

1. Examination of escape behaviour in freely moving animals with chronically implanted nerve cord electrodes has clarified the normal function of the giant fibres and the general organization of escape behaviour in the crayfish.

2. The giant fibres react almost exclusively to sudden stimuli; they do so at very short latency; and they produce rather stereotyped actions. Certain visual stimuli such as very rapidly approaching objects and rostral tactile stimuli such as taps to the anterior carapace or antennae fire the medial giants; caudal stimuli such as taps to the abdomen fire the lateral giants. In free animals even very weak stimuli often excite the giants.

3. Spontaneous tail flips and responses to more gradual stimuli are mediated by non-giant systems. When latencies can be meaningfully measured, they are at least an order of magnitude greater than those of giant-fibre reactions. Visual stimuli such as reaching for an animal or walking past its aquarium and somatic stimuli such as pinching or even cutting typically excite these systems.

4. Animals commonly escape with a sequence of tail flips (swimming). When they do this, the first flip of the sequence is determined as above, but subsequent flips are almost always mediated by non-giant systems. Both swimming and delayed (i.e. non-giant) escape involve a variety of phasic abdominal movements.

5. Experiments with animals whose nerve cords were cut at various levels indicate that non-giant escape either originates in or is strongly facilitated by the suboesophageal ganglion complex.

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