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EXTRINSIC MODULATION OF CRAYFISH ESCAPE BEHAVIOUR

BY FRANKLIN B. KRASNE*

Department of Psychology, University of California, Los Angeles, California 90024, U.S.A.

AND JEFFREY J. WINE

Department of Psychology, Stanford University, Stanford, California 94305, *U.S.A.*

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SUMMARY

Extrinsic systems were shown to control the excitability of the neurones which mediate tail-flip escape in the crayfish. Restraint suppresses the escape mediated by giant fibres and some, but not all, categories of non-giant mediated escape; autotomy of claws increases the excitability of non-giant mediated escape without affecting the lateral giant reflex.

The effects of restraint on the lateral giant reflex result from inhibition rather than reduced facilitation. The inhibition descends from thoracic and and higher levels, and the lateral giant escape command neurone appears to be its primary target. Inhibition may serve to shift the control of escape behaviour from short latency 'reflex' systems to more flexible 'voluntary' ones which can produce responses at times most opportune for successful escape.

INTRODUCTION

Animals seldom respond in fully predictable ways to a given stimulus. Variation in behavioural responsiveness can be regarded as the product of many learning and motivational processes, which have been studied at behavioural and gross physiological levels for many years. Recently, however, a great refinement in the analysis of behavioural variation has resulted from the establishment of neuronal circuit diagrams for a number of invertebrate behaviours. Once the neurones that mediate a given stimulus–response relationship are identified, it becomes possible to discover the precise loci and causes of variations in transmission through the neuronal network.

Behavioural modifications produced by past experience have received the most detailed attention in the form of several successful studies of short-term habituation in well analysed stimulus–response pathways (Kandel *et al.* 1970; Zucker, 1972*b*). In these systems habituation has been shown to be the consequence of depression at synapses within the stimulus–response pathways. In hierarchically organized nervous systems one also commonly finds behavioural variation that is produced by modulation of lower levels by functionally higher ones. Such modulation is believed to play an

* Reprint requests should be sent to Dr Krasne.

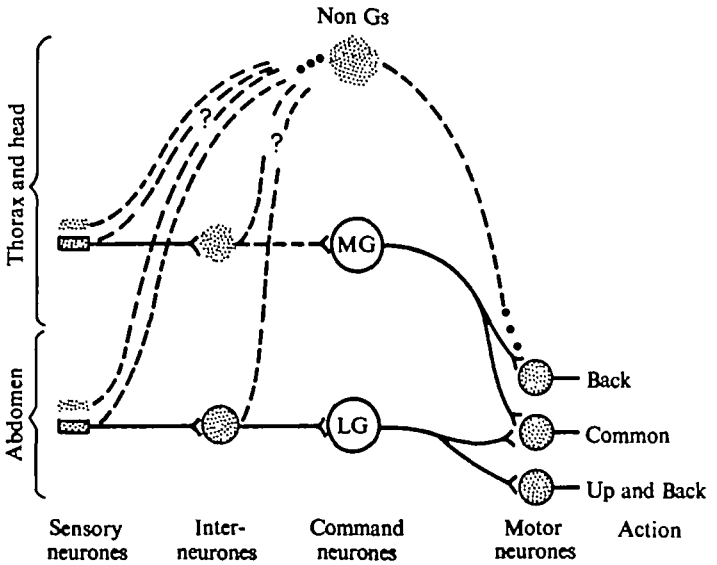

Fig. 1. Schematic representation of the neural circuitry involved in the crayfish escape responses. Identified neurones are shown as solid lines and inferred or conjectured neurones as dashed ones. Shading indicates that a population of neurones (in parallel) is represented. The LGs receive input via one tier of interneurones from abdominal tactile receptors (Zucker *et al.* 1971; Zucker, 1972*a*) and synapse with a set of motoneurones whose concerted action is to cause the animal's abdomen to pitch upward (Larimer *et al.* 1971; Wine & Krasne, 1972; Mittenthal & Wine, 1973). The MGs receive input from visual and tactile receptors of head and thorax via unidentified interneurone circuitry (drawn like the LG reflex for symmetry). The MGs synapse with a population of motoneurones which overlaps partially with those fed by LGs and which produce a backwardly directed dart. The non-giant circuitry prior to motoneurones is totally unidentified although non-giant neurones that evoke tail-flip behaviour have been identified in circumesophageal connectives (Atwood & Wiersma, 1967; Bowerman & Larimer, 1974). Some known connexions that are not important in this paper have been omitted.

important role in both higher invertebrates (Roeder, 1953; Rowell, 1970; Wiersma, 1970) and vertebrates. In this paper we document the existence of superordinate control on the circuitry which mediates tail-flip escape in the crayfish and begin to explore its circumstances and mechanisms of action.

Our current knowledge of the circuitry which mediates escape is summarized in Fig. 1. Two fixed action patterns are mediated by identified pairs of giant command interneurones; the medial giants (MGs) produce flips which propel the crayfish directly backwards, while the lateral giants (LGs) produce flips that pitch the crayfish forward. Tail-flips of more variable form and longer latency are mediated by unidentified, non-giant pathways (Wine & Krasne, 1972). In the circuit which includes the LG command cell (Fig. 1), representative neurones have been identified at every level from receptors to muscles, and synaptic transmission between each level has been characterized (Zucker *et al.* 1971; Zucker, 1972*a*, *b*, *c*). It is primarily the LG circuit for which analytical progress is now possible.

METHODS

Animals

Procambarus clarkii was obtained from local suppliers. Most animals were about 6·5 cm from rostrum to telson, but in a few experiments where we desired a higher threshold for escape behaviour animals about 8 cm in length were used.

Operative procedures

All operations were performed on animals cooled gradually to near freezing and then partially covered in crushed ice. Postoperatively, animals were placed in water at 1–3 °C and allowed to warm gradually to room temperature. Thereafter, animals were maintained at 18–20 °C in individual, aerated, 19 l aquaria.

Implanted electrodes for muscle or nerve cord recording consisted of pairs of Formvar insulated copper wires inserted either into the abdominal flexor muscles or near enough to the abdominal nerve cord to pick up giant axon potentials. The wires were threaded into hypodermic needles and bent back over the tip (Hanegan & Heath, 1970); the needles were then inserted into the abdomen at the dorsal junction of thorax and abdomen and pushed to the desired location. When the needles were withdrawn carefully, the bent wire electrodes caught on muscles and stayed in place, even during moulting. The wires were led to connectors on styrofoam floats and could be connected to amplifiers with minimal disturbance to the animals.

Transections of the nerve cord were made through small windows in the ventral cuticle. Where pieces of hard exoskeleton had to be removed, they were replaced and allowed to seal with congealed blood before returning animals to water. The two ends of cut nerve cords were seen to pull apart when severed, with the intact ventral artery bridging the gap.

Tests were run for at least a few weeks after operations and sometimes were continued for up to 1 month. Recovery from effects produced by nerve cord section did not occur within this period.

Stimuli and response scoring

Stimuli were chosen selectively to test the excitability of the various systems (Wine & Krasne, 1972). Phasic stimuli (taps) selectively elicit giant fibre-mediated escape; taps to the abdomen were used to test the LG reflex and taps to the head just behind the eyes were used to test the MG reflex. Non-giant mediated escape was tested by slowly moving a pair of forceps toward an animal and then gradually squeezing a leg or the abdomen.

Flips produced by rostal or caudal taps were presumed to be mediated by MGs or LGs, respectively. When electromyograms were available, only short latency (less than 30 msec) tail-flips were categorized as giant-mediated (Wine & Krasne, 1972). This criterion of latency was almost always met when responses were elicited by taps. Only those presumptive non-giant mediated flips evoked by visual approach, contacting the animal with forceps or pinching were counted; flips occurring only at release after a pinch were considered as failures to respond. Once an animal executed a tail-flip, the non-giant test trial was ended.

Taps were delivered by hand with a steel rod attached to a strain gauge which

measured the intensity of the stimulus and the time of contact. This provided a signal for oscilloscope triggering and permitted latency measurements. The abdomen was compressed with forceps having springy jaws which delivered approximately 50 g force per centimetre width of crayfish when entirely closed. Pinches to the legs were firm squeezes with forceps.

Testing procedures

Unless otherwise described, the effects of restraint were tested as follows: An animal was stimulated while free to move within its home aquarium (Trial I). It was then caught and stimulated while held between the experimenter's fingers (Trial II). Finally, it was released, allowed to regain its normal posture, and then stimulated once again (Trial III). The trials in a triplet were 30 sec to one min apart. About four triplets were run per day, half in a morning and half in an afternoon session, most animals being tested for 2–5 days. To quantitatively compare the effects of restraint after various treatments, 'relative percentage responsiveness during restraint' was defined as the percent responses on Trial II of all triplets divided by the mean of the percent responsiveness on Trials I and III of the same group of triplets.

In the experiments summarized in Table II a trial consisted of the following sequence: (1) grasping an animal either by carapace alone or by the carapace and the sides of the first five abdominal segments, (2) pausing 10 sec, (3) applying the test stimulus mildly (leg pinch or uropod pinch) and then, if no response occurred, increasing the strength gradually for up to 3 sec, (4) pausing for 10 sec during which spontaneous tail-flips were scored, (5) repeating the stimulus, (6) releasing the abdomen if it was being held and (7) releasing the animal. Four such trials on 12 animals were given each day (spaced over 8 h) with the type of restraint and stimulus being rotated until 30 trials had been run.

RESULTS

Manipulations which influence excitability of crayfish escape behaviour

Casual observation indicates that the escape tendencies of crayfish are extremely variable. On some occasions, a crayfish may tail-flip in response to seeing a slight movement at a distance from its aquarium, while on other occasions the same animal may be closely approached by the investigator without a reaction. Similarly, while animals will often escape from the slightest contact if one attempts to prod them, they sometimes ignore the stimulus, walk away, or even attack the offender with their pincers.

The extent of arousal, stage of moult cycle, past history of stimulation, the presence or absence of pincers, and degree of restraint are among the variables which we have observed to influence escape responsiveness. Other factors are undoubtedly important; for example, large animals and females carrying eggs or young have high escape thresholds. In this report we concentrate primarily on the effects of restraint, for it offers clear evidence that the escape reflexes are subject to modulatory control and provides a reliable and easily controlled effect that is convenient for further analysis.

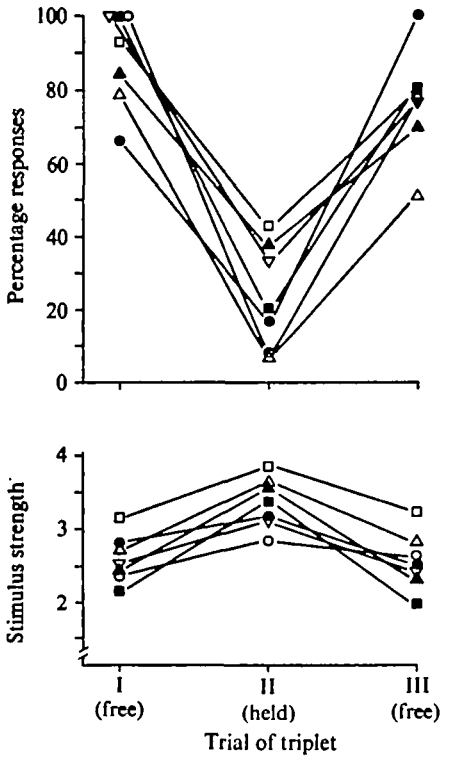

Fig. 2. Suppression of LG responses in animals held by the thorax in air. The animals were free on Trials I and III and held on Trial II. Each symbol refers to a different animal. Response scoring in this experiment was based on muscle potential latencies (see Methods). In this and all subsequent figures stimulus strength measures are on an arbitrary, but consistent scale.

Effects of restraint

The LG reflex was tested by tapping the abdomens of animals that were alternately free, held by their carapaces out of water, and free again. Escape responses were strongly and reversibly suppressed during restraint (Fig. 2). This suppression was not associated with any behaviour or posture that was obviously incompatible with tail-flips. Sometimes animals that were held by the carapace did curl their abdomens beneath themselves, escape responses being least frequent (mean of 11 %)* on such trials. However, this value is only slightly below that measured on trials in which the animals' abdomens were straight (mean of 15 %) or were forced into an extended position by the experimenter (mean of 15 %). These values are much lower than those obtained with the same animals when free (64 %).

The suppressive effects of restraint extend also to the other categories of escape. Fig. 3 shows suppression of both MG and non-giant-mediated flips. Giant-mediated responses were also suppressed, though not as markedly, when animals were held by the carapace under water (Table 1).

Although restraint greatly reduces the probability of tail-flips, the animal is not in a state of general behavioural inhibition. Animals held by the thorax often display stereotyped defence responses, gradually extend and flex their abdomens, or struggle.

* These figures are from a group of seven animals different from those used in the experiment of Fig. 2.

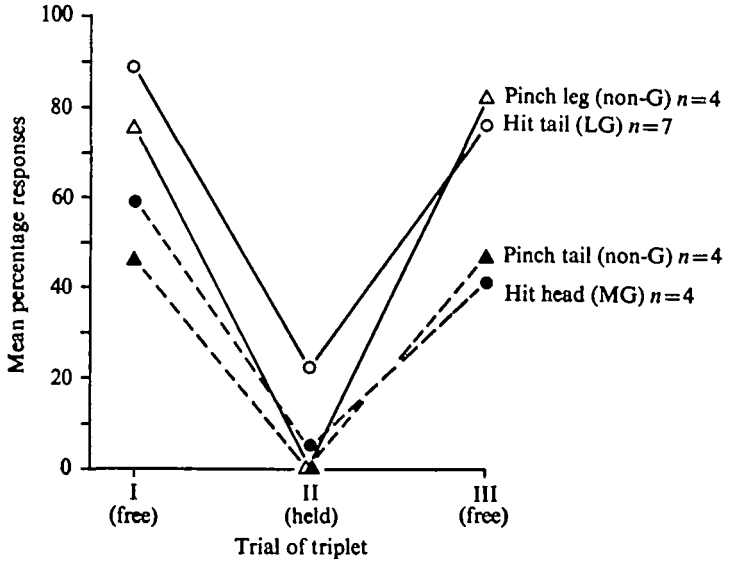

Fig. 3. Suppression of MG, LG, and non-giant responses during restraint. The number of animals tested in each condition (n) is indicated.

Table 1. *Mean relative percentage responsiveness during restraint (and proportion of animals showing suppression)*

	LG	MG
Held by carapace out of water	26% (7/7)	11% (4/4)
Held by carapace in water	74% (5/7)	10% (4/4)

Moreover, whereas tail-flips mediated by non-giant circuitry are severely suppressed in animals held by the thorax when pinches are delivered to the legs or abdomen, pinches to a uropod often evoke tail-flips that pull the uropod out of the jaws of the forceps. Animals held by the thorax also tend to spontaneously flip out of the experimenter's hand if his grasp loosens. These observations suggest that tail-flips which could be useful in ridding an animal of noxious stimulation might persist during restraint, while flips which would be useless are suppressed. Table 2 documents these effects. Contact with the uropods in an animal held by the thorax (row C) is obviously a quite effective stimulus, as is release of the abdomen in an animal that has been held by both abdomen and thorax (row K). Tail-flips to uropod pinches usually occur at initial contact or to a light squeeze; once a uropod is firmly grasped (so that the animal is unlikely to be able to free it by flipping), responses become much less likely though the stimulus is more intense (rows C *v.* D ($P < 0{\cdot}01$, two-tailed Wilcoxon signed ranks test) and H *vs.* I). Tail-flips also occurred frequently as the hold on an animal was released, particularly if the animal had been held by both thorax and abdomen (row L).

It could be that specific stimuli which evoke tail-flips during restraint in the above experiment are simply effective enough to overcome a generalized inhibition. Alternatively, maladaptive or useless responses might be *selectively* inhibited. In the latter case it might be expected that if an animal were held by both thorax *and* abdomen, sq

Table 2. *Non-giant escape responses to various stimuli during two configurations of restraint (see Methods)*

Type of restraint	Stimuli (and conditions for scoring a response)	Mean % responses	Animals out of 12 showing ≥ 10% responses	Mean no. of possibilities for response per animal
Held by thorax	A. Pinch leg	3	2	20
	B. Release leg (given no response to pinch)	1	0	18
	C. Touch or pinch uropod lightly	59	11	19
	D. Squeeze uropod firmly (given no prior response)	12	4	7
	E. Release uropod (given no re-response to pinch)	10	2	6
	F. Release animal (given that prior responding had terminated)	7	2	6
	G. Trials with 'spontaneous' responses	14	5	20
Held by thorax and abdomen	H. Touch or pinch uropod lightly	34	8	11
	I. Squeeze uropod firmly (given no prior response)	8	3	18
	J. Release uropod (given no response to pinch)	7	2	11
	K. Release abdomen (given that prior responding had terminated)	28	7	4
	L. Release animal (given that prior responding had terminated)	37	8	5
	M. Trials with 'spontaneous' responses	13	6	10

that it could not withdraw from uropod pinches by flexing its abdomen, then the escape reactions to such pinches would be inhibited. In fact, combined abdominal and thoracic restraint does considerably reduce tail-flip responses to uropod pinches (rows C *v.* H; $P < 0{\cdot}01$, two-tailed Wilcoxon signed ranks test). It seems unlikely that this is because inhibition is stronger when the abdomen and thorax are simultaneously held, because spontaneous flips (rows G and M) occur about equally in both conditions of restraint.

Effects of claw removal

When large individuals are threatened by another crayfish or by approach of an object, they usually do not escape. Instead, the animal faces the source of stimulation and raises and opens its pincers (the 'defence' posture). A common, but hitherto undocumented, observation is that escape is more readily elicited after the pincers are autotomized. We attempted to verify this phenomenon and to follow its time course for both giant and non-giant mediated escape in free animals. Stimuli for LG-mediated and non-giant-mediated escape were each given 9 times per day at 30 sec to 1 min intervals for 6 days before and 6 days after autotomy. No tests were made on the day of autotomy.

Following autotomy there was a pronounced increase in the probability of non-giant mediated escape (Fig. 4). Prior to autotomy, but after some habituation due to previous testing, most animals did not try to escape until one of their legs was actually

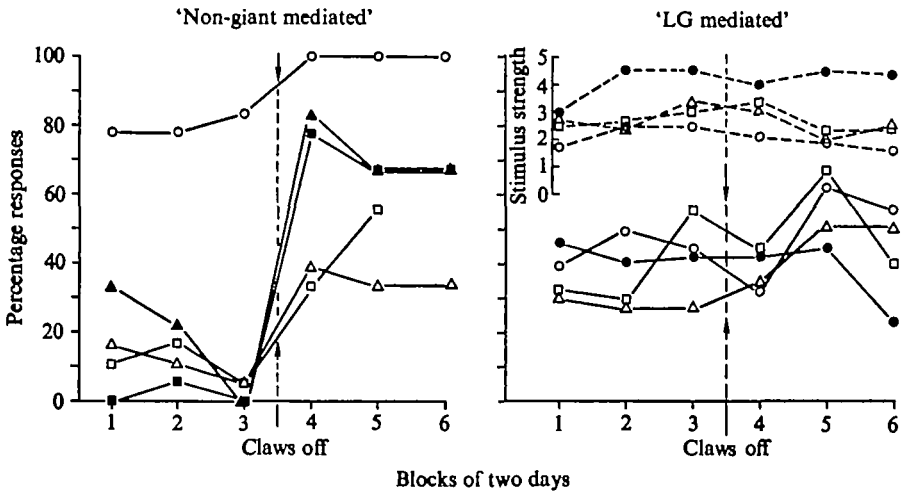

Fig. 4. Effects of claw removal on the excitability of escape. The left-hand graph shows results for non-giant mediated responses and the right hand graph results for LG-mediated responses (mean stimulus strengths are inserted as dashed curves at the top of the graph). Each symbol used for plotting points refers to a specific animal; the animals marked as open symbols were common to the two graphs. For non-giant tests, the ordinate gives the percentage of trials on which the animal escaped in response to approach or *initial* contact.

grasped or until they were released after being squeezed. Following autotomy, however, escape responses to visual approach or light touch became common (two-tailed t-test on the difference between the two days before and after autotomy, $P < 0{\cdot}02$). This increase was fully developed on the day after autotomy and was independent of agonistic interactions with other crayfish. In contrast, there was no statistically significant change in LG-mediated escape.

Origin of the suppression caused by restraint

Transection of the ventral nerve cord at the thoracic–abdominal junction abolishes the suppressive effect of restraint on LG excitability (Fig. 5). This is not simply because the thoracic carapace (by which normal animals were held in the experiments described above) is no longer in neural communication with the abdomen, for restraining a transected animal by grasping the abdomen is also without effect (Fig. 5) even though such treatment strongly suppresses the LG reflex of intact crayfish. Thus, suppression depends upon influences which descend to the abdominal ganglia from higher centres.

The origin of these influences was investigated by making cuts at several positions along the cord (Fig. 6). Removal of the supraesophageal ganglion (brain) caused a significant decrease in restraint-induced suppression of LG responses* (t-test, normal $v.$ brainless, $P < 0{\cdot}001$, two-tailed) but did not completely abolish that phenomenon (brainless $v.$ abdominal, $P < 0{\cdot}01$). This operation also produced a pronounced loss in the ability to suppress non-giant responses to leg pinches; relative percent responsiveness during restraint (see Methods) was 81 % as compared to 0 % in intact

* There were several indications in the data that ability to suppress recovered somewhat following the operation in this group.

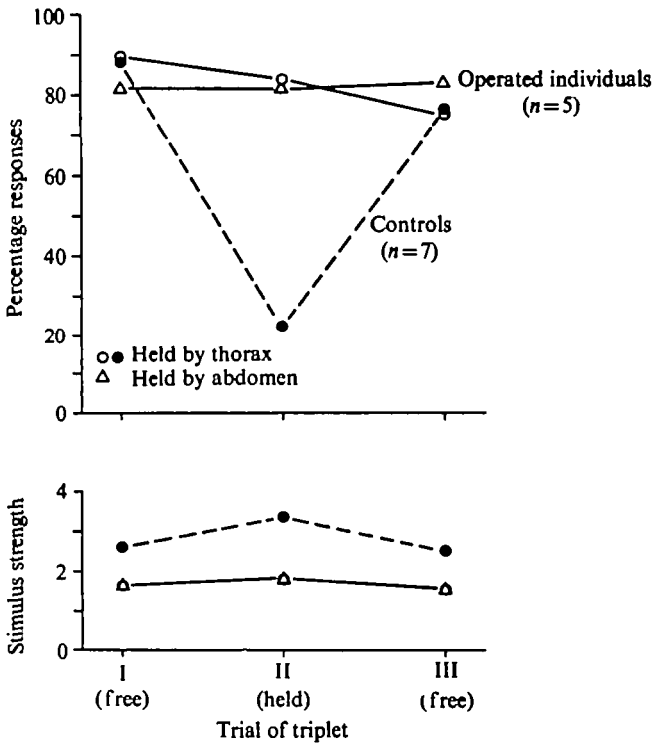

Fig. 5. Abolition of effect of restraint in animals with nerve cords severed at the thoracic–abdominal junction. Cuts were made either between the last thoracic and first abdominal ganglia or between the first and second abdominal ganglia. All animals had implanted muscle recording electrodes.

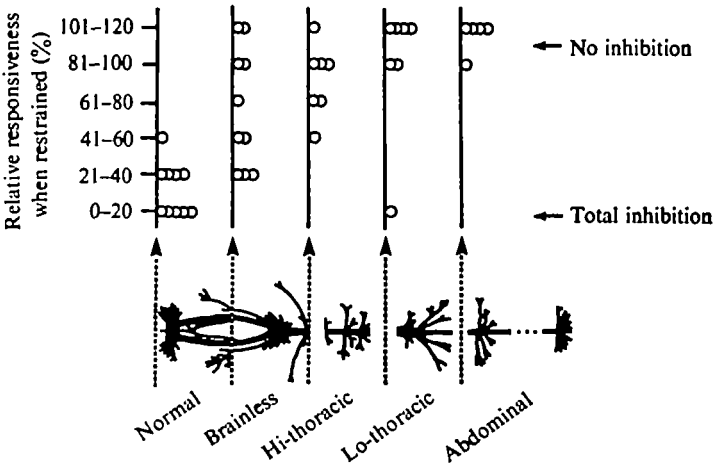

Fig. 6. Amount of inhibition of the LG reflex during restraint as a function of level of cord transection. Each dot represents the average level of responsiveness during restraint for an individual animal. See Methods for the definition of 'relative responsiveness'.

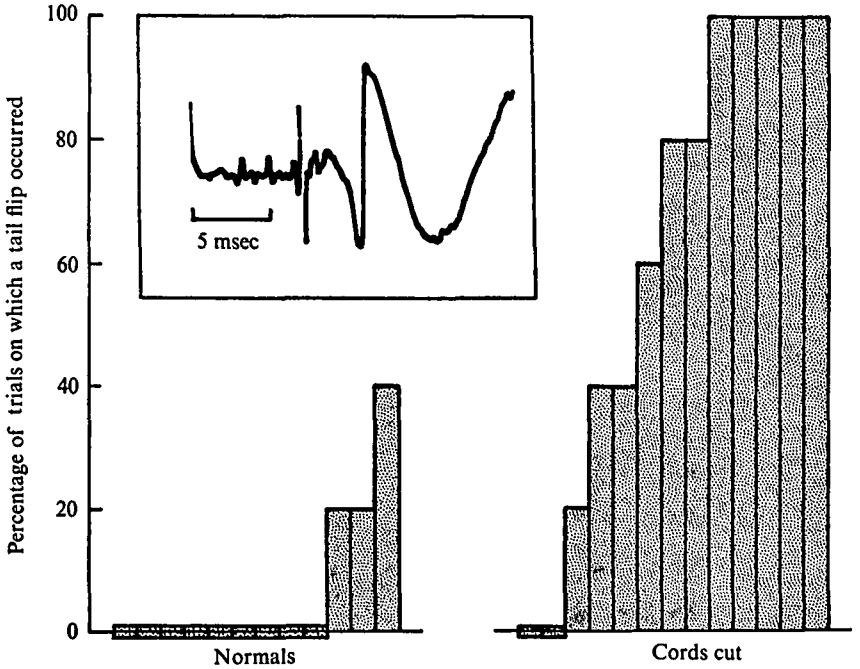

Fig. 7. Responses of restrained animals with intact or severed nerve cords to a uniform tap stimulus. Each bar represents the percentage responses from five trials on an individual crayfish. Inset is an oscilloscope trace of the abdominal nerve cord's electrical activity following a tap to the side at the start of the trace; LG activity at about 7 msec is followed by flexor muscle activity at about 10 msec. (From Wine & Krasne (1969); reprinted with permission of the American Psychological Association.)

animals (cut *v.* normals, $P < 0{\cdot}001$). Removal of additional rostral nervous tissue led to a progressive loss of restraint-induced suppression of LG-mediated tail-flips; little or no suppression remained after low thoracic or thoracic–abdominal cuts. The one aberrant low-thoracic animal of Fig. 6, which was studied extensively, not only maintained an ability to suppress but also appeared normal in its ability successfully to extricate itself from its old exoskeleton at the time of moult. We suspected that central regeneration might have occurred, but a terminal examination showed complete separation of the cord.

Thus, the ability to suppress LG responses during restraint seems to arise in a distributed fashion but to depend heavily upon the integrity of the supraesophageal and subesophageal ganglia.

Sign of descending influence

An influence that modulates the threshold of a response could operate either by inhibiting a response of high intrinsic excitability or by facilitating a response of low excitability. If inhibition were responsible for the modulation of the LG reflex, then cord transection between thorax and abdomen should produce a high level of LG excitability (comparable to or greater than that seen in a free, intact animal), whereas if modulation were achieved via descending facilitation, then transection should produce a condition of low excitability (comparable to or less than that seen in a restrained, intact crayfish).

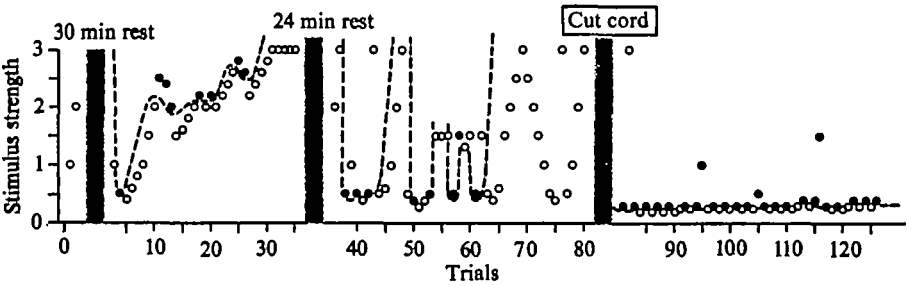

Fig. 8. LG reflex threshold during maintained restraint before and after cutting the nerve cord. Single shocks to a second root of the third abdominal ganglion were given at 30 sec intervals. Each point represents a single trial. The ordinate value gives the shock intensity; filled points indicate that the LGs fired, while open points indicate that they did not. The dashed curve gives a subjective interpretation of the moment-to-moment stimulus threshold for LG firing based upon the data points. Note that upon cutting the cord between thorax and abdomen the threshold dropped to slightly beneath the lowest level seen while the nerve cord was intact.

A systematic comparison of transected and normal animals during restraint (Fig. 7) showed that the excitability of LG escape is markedly greater in transected animals that have lost the ability to suppress than in normal ones. Furthermore, Fig. 5 illustrates that free crayfish with transected nerve cords have lower thresholds than control animals. Hence, the reduced likelihood of escape during restraint is due to an active inhibitory influence operating on a reflex of high intrinsic excitability.

This conclusion also follows from experiments in which acute transections of the ventral nerve cord were performed on restrained, minimally dissected animals. Electrical shocks to an afferent root provided precisely controlled stimuli. A running measure of the stimulus threshold for LG firing was obtained by varying shock intensity from trial to trial so as to bracket continually the LG threshold. Cutting the cord always caused the threshold to drop immediately to a low and steady value. In some cases, there were wide and frequent swings of excitability while the cord was intact, but these never fell below the level seen after cord transection (Fig. 8). This suggests that the LG threshold of the cut animals is the lowest which the system can achieve. However, it is also possible that facilitatory circuits exist, but are simply not active in experiments of this kind. The wide swings in excitability also demonstrate that the modulating system is graded, rather than all-or-none.

The decline in threshold in these experiments occurred within a few seconds of cord transection. This eliminates the possibility that the apparent inhibition is due to descending excitatory bombardment of those labile synapses in the LG pathway that are known to be responsible for short-term habituation, for at least a few minutes would be required for excitability to rise following removal of such bombardment (Krasne, 1969; Zucker, 1972*b*).

Finally, it appears that the inhibitory system has primarily an ipsilateral projection. Seventeen experiments were conducted as above, except that LG thresholds were determined for both left and right root stimulation, and then the effects of cutting a single hemiconnective were observed. Nine experiments were discarded either because the animal never responded ($N = 6$) or did not show inhibition (that is, always responded to low intensity root shocks). In the remaining eight experiments,

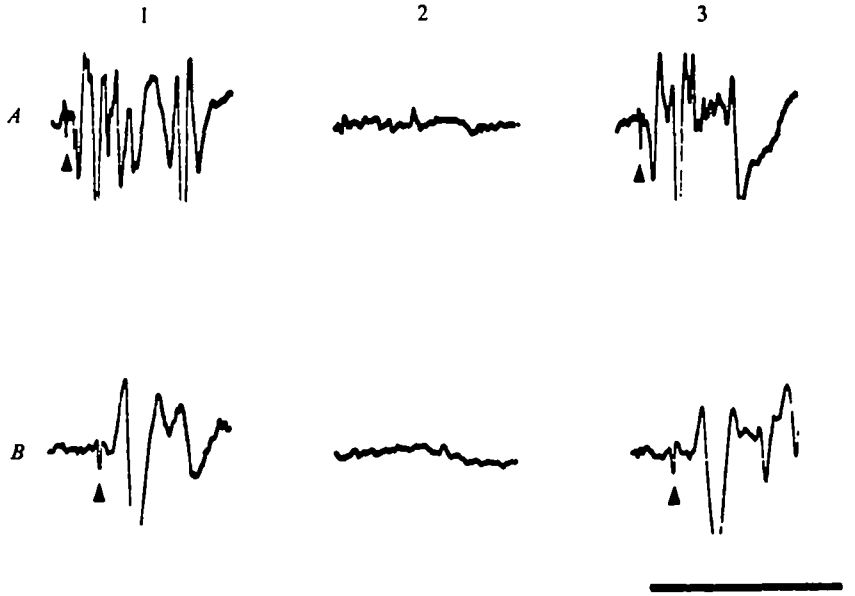

Fig. 9. LG and muscle potential recordings in free and restrained animals. Recordings during two triplets (*A*, *B*) from two different animals are shown. In each case the animal was free on Trials 1 and 3 and restrained on Trial 2. LG spikes (indicated by solid triangles) are followed by muscle potentials. Scale: *A*, 50 msec.; *B*, 20 msec.

Table 3. *Chronic recordings of LG and muscle potentials in three animals on free and restrained trials*

Event	Percent occurrence (relative frequency)	
	Free	Restrained
Unambiguous giant fibre spike	85% (64/75)	27% (9/33)
Tail-flip *given* that LG fired	100%	100%
LG-evoked muscle potential small or delayed	5%	45%

transection of a hemiconnective caused a marked reduction of threshold only for stimuli ipsilateral to the cut.

Which portions of the LG reflex circuit are inhibited by restraint?

The LG reflex is mediated by a trisynaptic reflex arc (Fig. 1). Sensory neurones converge on interneurones which in turn converge on the LG command neurone; the LG then makes divergent connexions to the fast flexor motoneurones (Wiersma, 1947; Selverston & Remler, 1972; Zucker, 1972*a*). *All* of these elements are known to be phasically inhibited by circuits intrinsic to the abdominal cord during a tail flip (Furshpan & Potter, 1959; Roberts, 1968; Wine, 1971; Mittenthal & Wine, 1973; Krasne & Bryan, 1973).

Restraint-induced inhibition of the LG reflex must include inhibition of elements afferent to the LGs or of the LGs themselves, because during restraint the probability

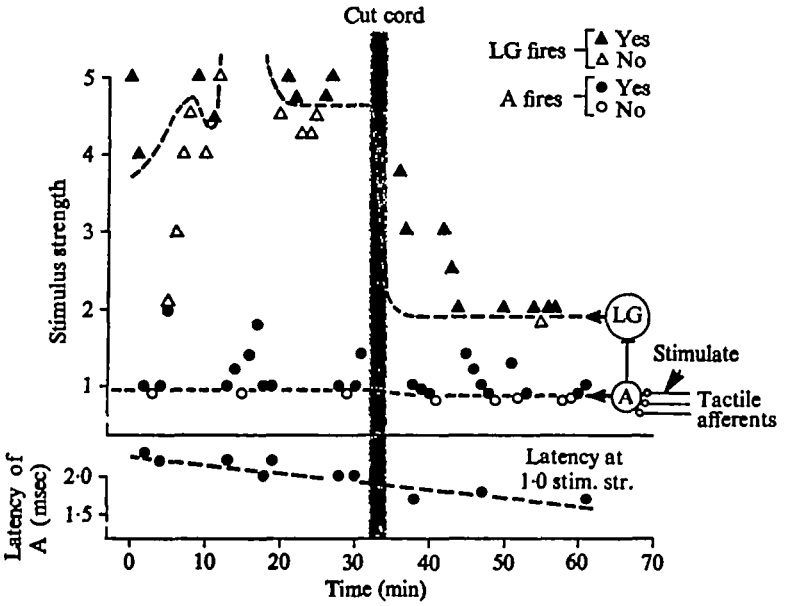

Fig. 10. Thresholds for LG and interneurone A firing in restrained crayfish before and after cutting the nerve cord. The method was essentially as in Fig. 8 except that the LG and interneurone A were tested alternately, and the stimuli were shocks to the second and third roots of the last abdominal ganglion. The measurements plotted in the upper graph give a slight suggestion that interneurone A's threshold might have decreased very slightly at the time of cord section, but measurements of interneurone A's firing latency at constant stimulus strength, plotted below, show that this decrease was in fact the manifestation of a gradual decline of interneurone A's threshold throughout the experiment.

of LG firing (Fig. 9; Table 3) is reduced, yet when the LGs do fire, tail flips are always observed. However, when the LGs fired during restraint, delayed or reduced muscle potentials were often seen (Table 3), suggesting that there may be a partial inhibition of the efferent portion of the LG circuit. We cannot yet rule out the alternative possibility that the difference in muscle potentials is caused by the different feedback that could occur when the animal flips its tail in air.

The LGs could in principle be prevented from firing by inhibition at three loci: the LGs themselves, the sensory interneurones, and the afferent terminals (presynaptic inhibition). We have been unable to maintain the inhibition resulting from restraint in animals which were sufficiently dissected to permit intracellular recordings from either the LGs or sensory interneurones. In minimally dissected preparations, however, spikes in some of the large sensory interneurones can be recorded with gross electrodes while the inhibitory circuits are still operative. In particular, the largest of these, interneurone A (Zucker *et al.* 1971) can be identified by its large spikes, location in the cord, ipsilateral receptive field (mainly on the dorsal telson), low threshold to phasic stimuli such as taps to the preparation dish, and low threshold and short latency to shocks to afferent axons in ipsilateral roots 1–5 of the 6th ganglion (Kennedy, 1971, and personal communication). We took advantage of these properties to determine the locus of inhibition in the sensory – LG pathway. Roots 2 and 3 of the last abdominal ganglion were shocked at one per minute, and the stimulus level was set near the expected thresholds for either interneurone A or LG on alternate

trials. By varying the levels slightly from test to test, the stimulus thresholds for firing each neurone could be followed, as illustrated on the left of Fig. 10. After thresholds were established, the cord was transected between abdomen and thorax, thus releasing the LG reflex from descending inhibition. As shown on the right of Fig. 10 the threshold for the LG dropped dramatically, while that for interneurone A remained constant except for a gradual increase of excitability (noticeable mainly as a latency change) which continued *throughout* the experiment. Since the recurrent inhibition previously described (Krasne & Bryan, 1973) usually increases the threshold for interneurone A by more than 100% when it is tested in this same manner, we feel reasonably sure that the sensitivity of our measuring procedure was such that a change of excitability at the first synapse would have been detected if it were present. Therefore, in so far as we may take interneurone A as a typical of interneurones in the LG circuit, we may conclude that the tonic descending inhibition produced by restraint operates between interneurones and command fibres and not between afferents and interneurones.

DISCUSSION

The purpose of this research was to document the existence of extrinsic modulation of the neuronal circuits which mediate crayfish escape responses and to explore the mechanisms of the modulation.

Three main effects have been established: (1) holding an animal by the thorax suppresses LG and MG responses; (2) such restraint suppresses some kinds of non-giant mediated escape, but certain stimuli remain effective and non-giant tail flips may also occur spontaneously; (3) removal of claws selectively increases reactivity of non-giant mediated escape, but does not appreciably affect the excitability of the LG system.

Functional interpretation

While we can only speculate on the functional significance of these results, hypotheses as to their meaning are helpful for guiding future work and for interpreting details of the way circuitry is organized.

Restraint-induced inhibition

Variations in behavioural or neuronal responses of arthropods have been attributed to arousal (e.g. Wiersma, 1970; Rowell, 1970; Taylor, 1970), and Rowell has specifically suggested that arthropods may possess a unitary arousal system with widespread inputs and outputs analogous to the reticular activating system of mammals. While an activating system may influence crayfish escape behaviour, the modulatory effects reviewed above do not seem to be unitary. Moreover, we have often observed that the first tap delivered to a quiescent crayfish appears to alert it without causing escape, while subsequent stimuli of the same intensity do evoke escape. Therefore, if there is a relationship between state of arousal and escape response excitability, it seems to be a positive one. Yet during restraint-induced inhibition, animals often struggle and show general indications of excitement. Hence, the effects of restraint cannot be conveniently explained in terms of arousal.

A second kind of modulation is that involved in coordinating incompatible responses. For example, righting and withdrawal behaviours in the carnivorous marin

gastropod *Pleurobranchaea* are suppressed by feeding behaviour (Davis & Mpitsos, 1971; Davis *et al.* 1974); feeding, swimming escape, and to some extent protective withdrawal in the swimming anemone *Stomphia* are mutually inhibitory to one another (Ross, 1964; Ross & Sutton, 1964); and LG and MG escape response pathways in crayfish are inhibited when a prior escape response is in progress.

Inhibition by competing responses cannot be solely responsible for the effects of restraint, because even completely quiescent animals fail to respond. However, mutual inhibition between behavioural systems may also take place in circuitry which precedes decision or command nuerones; in behavioural terms, this would mean that a given behaviour is inhibited in anticipation of a competing response – no overt act need occur. For example, application of food juice to satiated *Pleurobranchaea* does not lead to feeding responses, but righting behaviour is nevertheless suppressed much as it would be during overt feeding (Davis *et al.* 1974). In this context the occurrence of spontaneous and certain categories of evoked non-giant escape reactions in restrained crayfish (Table 2) suggests the possibility that circuitry responsible for non-giant escape may be active during restraint and may drive the inhibition of giant-mediated escape that we observe. The control of escape would thus be shifted away from systems obliged to produce short latency, stereotyped responses upon receipt of specific releasing stimuli to systems that seem to integrate a wider range of factors in order to produce responses at times most opportune for successful escape. In essence, we are suggesting that restraint causes an adaptive shift from 'reflex' to 'volitional' control of escape behaviour.

Observation of crayfish in large tanks indicates that such shifts occur during fights. As the animals grapple with one another the probability of phasic tactile stimulation is high, but most tail flips seem to occur, not as reactions to these stimuli, but rather to more subtle cues that are not as yet obvious.

Autotomy of chelipeds

Autotomy is a common defence mechanism among crustaceans. Increased excitability of escape systems should be advantageous to an animal deprived of its major means of defence. Our studies show that escape excitability does increase following autotomy and that this occurs rapidly and requires no experience with other crayfish. This automatic readjustment might well have evolved along with the autotomy reflex itself.

Only non-giant mediated escape appears to be facilitated by autonomy. Although this surprised us, in retrospect it seems understandable. Defence and non-giant mediated escape are alternative responses used in gradually developing threat situations where an animal has time to evaluate stimuli and test the success of defence. It is, thus, plausible that these competing strategies might inhibit one another and the rise of non-giant escape excitability after autotomy be due to a drop in defensive behaviour. By contrast, optimal stimuli for giant-mediated escape, like those used in our tests, may trigger giant fibre firing before defence can even be 'considered' and, therefore, before inhibition by active defence circuitry could arise. Consequently, no matter what inhibitory relations there may be between defence and escape, there would be little chance for defence to compete with escape on giant-mediated escape test trials, and thus, no release from inhibition would be seen after autotomy. Moreover, intact animals sometimes defended as the stimulator was brought into range and then

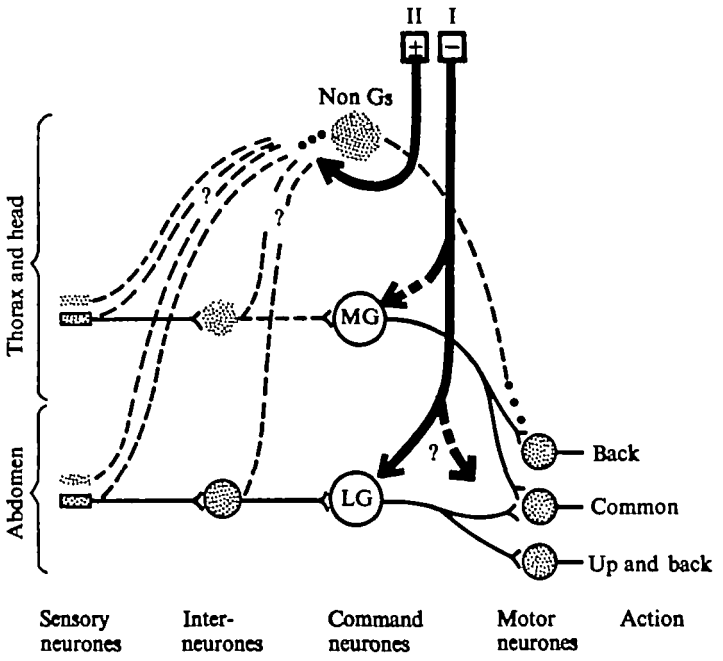

Fig. 11. Extrinsic modulatory circuitry. The circuit of Fig. 1 with Pathways I and II (see text) added as bold lines. The target of Pathway I in the MG reflex is conjectured to be the same as in the LG reflex. The dashed line pointing toward motor neurones indicates the possibility (see Results) that the inhibition also affects command-to-motor neurone transmission.

made a giant-mediated escape when tapped, which suggests that giant-mediated escape may not be inhibited even when defence circuitry *is* active. It may be adaptive for giant-mediated escape to remain consistently excitable independent of the possibility or the occurrence of defence.

Organization of modulatory system

Number of separable effects

The joint suppression of LG and MG reflexes during restraint suggests the existence of an inhibitory pathway that is activated by restraint and influences at least the giant fibre reflexes (Pathway I of Fig. 11).

Restraint-induced inhibition of non-giant escape is more complicated, because the particular stimuli which loose their ability to evoke non-giant escape depend upon the way in which the animal is restrained. Two interpretations are possible. Pathway I might exert an inhibitory influence uniformly on all escape behaviour, but under certain circumstances this inhibition might be overridden for some kinds of stimuli that can evoke non-giant mediated escape. Alternatively, inhibition of non-giant mediated escape might result from the operation of a rather complex system of special inhibitory pathways. Excitability of non-giant mediated escape is also selectively enhanced as the result of autotomy. Whether this is due to institution of a tonic excitatory or removal of a tonic inhibitory influence that is confined to non-giant systems or is due to loss of inhibition by competing defence response circuitry as suggested by the discussion in the previous section is not known. For schemati

convenience we have merely indicated the various effects that we have observed on non-giant escape as 'Pathway II' (Fig. 11) without intending any specific conclusions or hypotheses.

Level of influence

The observation that restraint-induced inhibition of the LG pathway does not involve inhibition at the first synapse requires confirmation for additional first-order interneurones. If the tactile interneurones which synapse on the LGs also feed information to decision networks for other behavioural acts, then it would be reasonable for inhibition to be confined to the response ends of the giant-fibre pathways.

This situation contrasts with several other examples of modulation in the crayfish, such as the recurrent inhibition of the LG pathway that occurs during escape. During a tail-flip, inhibition at the first synapse is necessary to prevent habituation of the LG reflex to self-produced stimuli (Krasne & Bryan, 1973). Furthermore, it should not result in loss of information, because the firing of tactile receptors that are being whipped through the water is probably meaningless. The sensitive 'water vibration receptor interneurone' (C4 of Wiersma & Mill, 1965) studied in detail by Taylor (1970) is also inhibited in anticipation of and during vigorous appendage movements.

Recurrent inhibition and descending 'inhibition of restraint' are but two out of a presumably larger number of effects that modulate escape reflex excitability. Indeed, preliminary data suggest that certain movements are associated with inhibition of the *first* synapse of the LG pathway and that under some circumstances descending facilitation can drive LG reflex excitability above the levels seen in the isolated abdominal cord. As more modulatory effects are characterized, we can question how they in turn are interrelated. Do they share final common paths? Do they have significant mutual interactions? Are they hierarchically organized? The answers to such questions should significantly advance our understanding of the control circuitry which determines how an animal will act in a given situation.

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