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# CRAYFISH ESCAPE BEHAVIOUR: COMMANDS FOR FAST MOVEMENT INHIBIT POSTURAL TONE AND REFLEXES, AND PREVENT HABITUATION OF SLOW REFLEXES

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## SUMMARY

Organized behaviour requires central neural mechanisms to prevent the simultaneous occurrence of incompatible movements. We investigated neural pathways in crayfish that suppress slow flexion of the abdomen during rapid flexions ('tailflips') produced by a separate set of muscles. The slow flexors are innervated in each half segment of the abdomen by five motor neurones and one peripheral inhibitor. In isolated preparations of the abdominal nervous system, stimulation of identified command neurones, which trigger tailflips in intact animals, inhibited spontaneous activity in the motor neurones to the slow flexors and excited the peripheral inhibitor. These effects are mediated by a population of interganglionic interneurones interposed between the command cells and the slow flexor efferents.

Slow flexor reflexes also were inhibited by escape commands. This inhibition includes pathways that act upon early stages of sensory input. As a result, habituation of reflexes, which normally is produced by repeated stimulation, is abolished if each sensory stimulus is preceded by a burst of impulses in the command neurone.

## INTRODUCTION

An understanding of interactions among the systems underlying different behavioural elements of an animal is crucial to any explanation of the behaviour of that animal. This principle was one rationale for the study of interactions among spinal reflexes (Sherrington, 1906), and among oscillatory behavioural elements like fin movements and respiration (von Holst, 1937, 1939). Ethologists emphasize that interactions also occur during more complex behaviour. For example, displacement activities, threat behaviour and aspects of courtship and other displays have been interpreted as consequences of inhibition and disinhibition among behavioural systems (e.g. Iersel & Bol, 1958; Rowell, 1961). It may be that neural mechanisms that mediate behavioural interactions are common to behaviours of differing complexity.

Study of behavioural interactions is now being made at the cellular level (see, for example, Stein, 1971; Burrows, 1975*a*, *b*; Koester *et al.* 1974; Kovac & Davis, 1977; Mulloney, 1977). The present research investigates interactions at the cellular level between two neuromuscular systems in the crayfish abdomen: the postural flexor system and the escape flexor system. Both systems act at the same points but are behaviourally incompatible in that they act at strikingly different speeds.

The neural basis of each behaviour is already known in some detail. The crayfish abdomen consists of six segments hinged to move mainly in the sagittal plane. The postural flexor muscles consist of thin sheets of slow muscle. In each half segment they are innervated by a thin nerve containing five small, tonically active motor neurones and a peripheral inhibitor. The fast flexor muscles that power the 'tailflip' of the escape response are massive, spiralling muscles, innervated in each half-segment by six to ten large motor neurones and a peripheral inhibitor (Takeda & Kennedy, 1964; Selverston & Remler, 1972; Mittenthal & Wine, 1978). The large flexor motor neurones are silent except during tailflips.

The two flexor systems are very different in both their time constants and frequency of use. Peak tension in fast flexors has a latency of 50 ms, whereas the *onset* of tension in the tonic muscles has a latency of about 100 ms (Roberts, 1968*b*) and peak tension may require several seconds (Evoy, Kennedy & Wilson, 1967). In adults the tailflip motor circuitry may be inactive for days, depending on the animal's environment, while in contrast the most active motor neurones in the tonic system can fire 12–17 impulses/s (Kennedy, Evoy & Fields, 1966) or over a million impulses a day.

These contrasts and the apparent independence of the parallel motor systems have led to independent investigations of them. Only two brief attempts to discover interactions have previously been reported. In one, antidromic stimulation of fast flexor motor neurones had no effect on the tonic motor neurones (Evoy *et al.* 1967), but we now know that antidromic impulses may fail to invade the synaptic regions of neurones (Mulloney & Selverston, 1972). In the other study, impulses in the giant interneurones that command tailflips were reported to cause a late, weak discharge in tonic motor neurones in addition to their earlier and more powerful excitation of the fast ones (Roberts, 1968*b*).

In this paper we study the response of the postural flexor system to stimulation of the tailflip command neurones. Unlike Roberts (1968*b*), we find that the main effect is inhibitory and is directed at multiple levels of the postural circuitry, including receptors, central neurones, and muscles. The command neurones have been previously shown to produce multiple level inhibition of the fast flexor and extensor systems (Roberts, 1968*a*, *b*; Wine, 1971, 1977*a*, *b*; Wine & Mistick, 1977; Wine & Hagiwara, 1978; Krasne, Wine & Kramer, 1977).

## METHODS

*Animals*. Experiments were performed on crayfish (*Procambarus clarkii*) measuring approximately 7 cm from rostrum to telson. The animals were obtained commercially and maintained in tanks at approximately 18–20 °C. Each crayfish was immobilized by slowly cooling to 3–5 °C, after which the abdomen was isolated and pinned to a dissection dish filled with cold, oxygenated van Harreveld's solution (van Harreveld, 1936), in which the bicarbonate buffer was replaced with Trizma at pH = 7·2. A strip of the ventral cuticle and ribs was removed to expose the ventral nerve cord. The main artery and excess connective tissue on the ventral surface of the nerve cord were removed, and the cord isolated by cutting all roots. The roots were kept as long as possible, especially the superficial branches of the 3rd roots (R3s), which contain the axons of the tonic flexor motor neurones. The cord was transferred to a Sylgard-lined

preparation dish and pinned out for recording. For muscle recordings, the cord was left in the abdomen and the main branches of the 3rd roots, which contain the axons of the phasic flexor motor neurones, were cut to prevent twitching. The ventral cuticle of a segment was removed to expose the tonic flexor muscle fibres.

*Stimulation and recording.* We used glass suction electrodes for extracellular recording and stimulation, and glass microelectrodes, filled with either 3 M-KCl or 4 M-K acetate for intracellular recording and stimulation.

The axons of the command neurones (Wiersma, 1938) that mediate the crayfish escape response comprise a pair of lateral giant (LG) axons and a pair of medial giant (MG) axons. They take up the whole dorsal surface of the nerve cord, each axon and its sheathing being about a quarter of the diameter of the cord. Selective stimulation was ensured by using a suction electrode with a tip diameter smaller than the axon, by using just threshold stimuli, and by monitoring the cord for impulses from other neurones evoked below the command neurone's threshold. Intracellular stimulation was used on two occasions to confirm the extracellular results.

After finding that stimulating the MG and LG axons had equivalent effects on the tonic motor system, we used the LG axons in most experiments because they are larger than the MG axons in the abdomen and so are easier to stimulate selectively. When we wanted to restrict the giant axon impulse to one portion of the nervous system, however, we used the MG axons because, unlike LG axons, they are not cross-connected in the abdomen. Each MG axon has almost symmetrical bilateral outputs (Selverston & Remler, 1972). This permitted us to transect one MG and then compare its effects with an intact MG (Roberts, 1968*a*). LG axons are cross-connected in every abdominal segment, so that an impulse initiated anywhere in them reaches both sides of all ganglia.

Single impulses in any giant axon usually produced weak and variable effects on the slow flexor system, but reliable effects were seen with brief trains. We adopted as a standard stimulus a train of five 0·1 ms pulses at frequencies of 165 to 200 Hz (i.e. 20–25 ms trains), and adjusted the stimulus intensity to a level that just drove the giant axons one-to-one. The use of exaggerated stimuli may be necessary to reveal connexions in depressed nervous systems such as the isolated abdominal cord, where even monosynaptic electrical transmission appears to be weakened (cf. Furshpan & Potter, 1959). However, trains of the length and frequency we used have been recorded in intact, freely moving crayfish in response to natural stimuli (Wine & Krasne, 1972).

*Identification and characterization of individual tonic flexor efferents.* Extracellularly recorded impulses of the six axons in the nerve leading to the slow flexors could be assigned to the individual axons on the basis of recorded amplitude (Fig. 1). Soma size, axon diameter and extracellular spike size are directly related; the cells are labelled according to increasing size from f1 to f6 (Kennedy & Takeda, 1965; Wine, Mittenthal & Kennedy, 1974). The second largest cell, f5, is the peripheral inhibitor. In isolated abdominal nervous systems, all of the slow flexor efferents are tonically active except f6 (Kennedy *et al.* 1966).

*Data collection and construction of histograms.* Recordings were made conventionally and photographed on an oscilloscope screen. Histograms of tonic motor impulses, with sample times of 10 or 25 ms, were constructed by hand. Samples of spontaneous activity of the motor neurones were taken before each trial and were used to establish

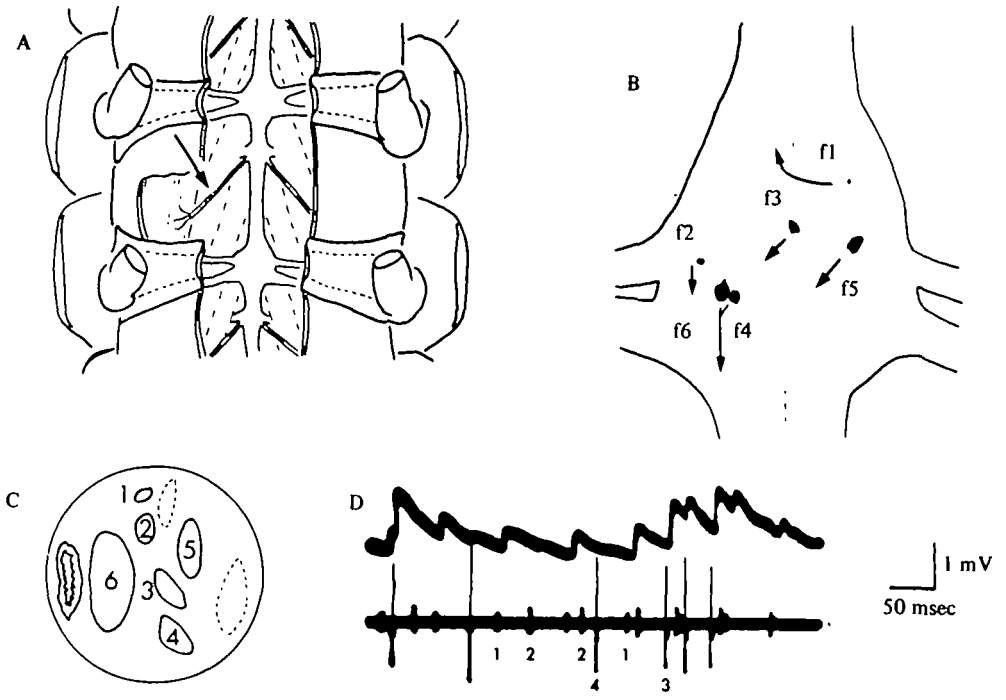


Fig. 1. The tonic flexor system. (A) A ventral view of segments 3 and 4 of the abdomen, with the swimmerets and a strip of cuticle cut away to expose the nerve cord and muscles. The thin sheet of superficial muscles is innervated by a fine, superficial branch of Root 3 (arrow) in each hemi-segment. (B) Soma map of tonic motor neurones in abdominal ganglion 2. Six cells are always filled by cobalt backfills of the thin branch of Root 3. Arrows show the course taken by the axons. (C) Cross section of the thin nerve to tonic muscles, showing six main axons and two bundles (outlines dotted) of very fine, unidentified axons. The axon diameters correlate well with soma diameters. (D) Identification of units by impulse amplitude and correlated synaptic potentials in the polyinnervated tonic muscle fibres. Only the 4 smaller cells are active in this record. A after Evoy & Kennedy (1967); B after Wine, Mittenthal & Kennedy (1974); C after Sokolove & Tatton (1975).

baseline activity. In any given preparation, confusion can occur between the identification of f1 and f2 and of f3 and f4 since the impulses are similar in both amplitude and activity. Therefore, we sometimes combined the data from either pair of these neurones. This was justified since in all the cases in which all six efferents were clearly distinguishable, f1 and f2 responses were very similar, as were the responses of f3 and f4.

## RESULTS

### *Lateral giant impulses excite the peripheral inhibitor (f5)*

The most obvious effect of LG impulses on the tonic system is to excite the peripheral inhibitor (f5). Excitation of f5 by brief LG trains was quantified by stimulating the LG axons to produce a train of five impulses, as described in the Methods. The inhibitor, which has a low rate of spontaneous activity in the isolated nervous system, was recruited during the stimulus train (Figs. 2, 3) and in one preparation was usually fired by a single LG impulse. The inhibitor was excited in every

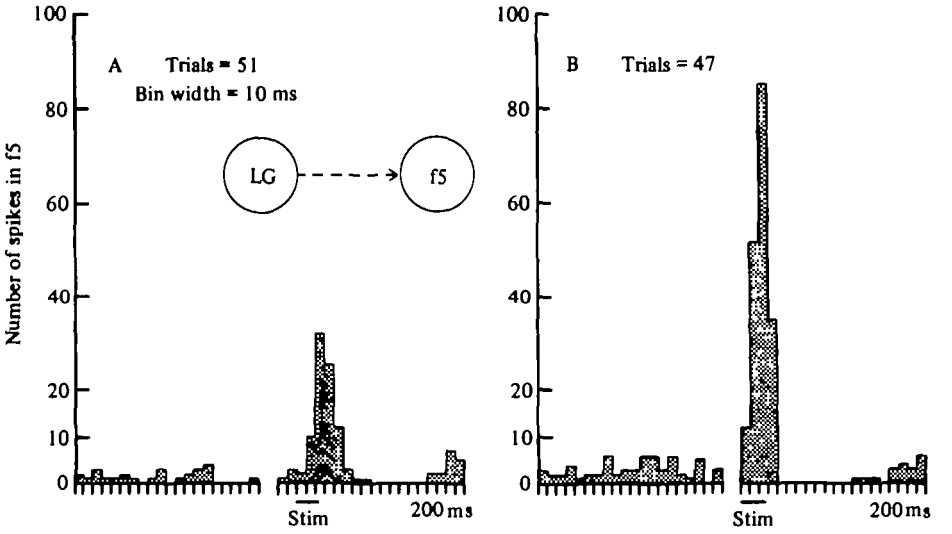


Fig. 2. Excitation of f5 by LG impulses. Histograms for two different animals, showing weak (A) and strong (B) responses. A period of reduced firing follows the LG-initiated bursts. The samples for spontaneous activity in f5 were taken at least 5 s after the activation of f5 by the LGs.

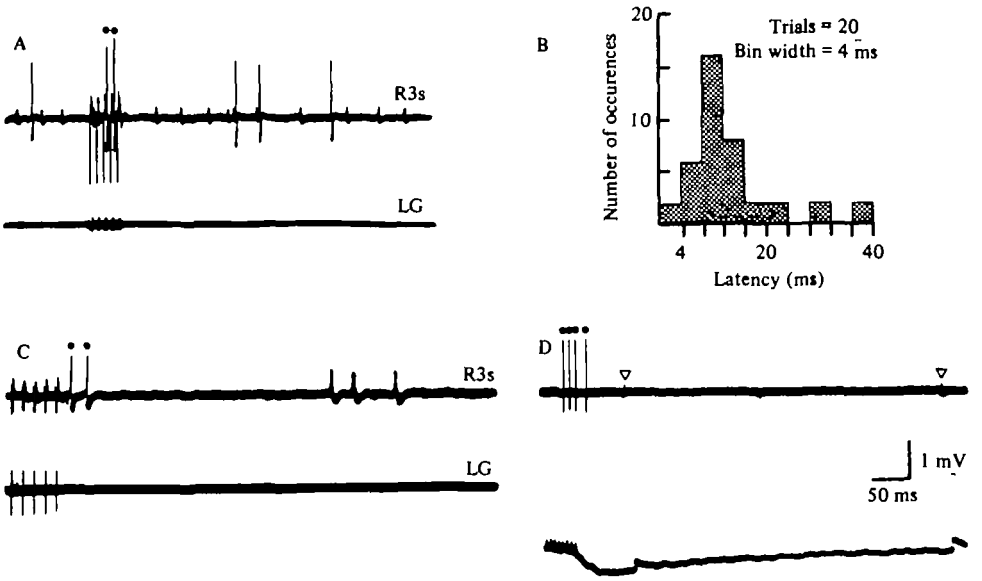


Fig. 3. Identification of f5 and details of its activation by LG impulses. (A) A train of LG impulses at 200 Hz (bottom trace) causes a pair of impulses in f5 (top trace, dots). (B) Latency of the f5 spike in an animal in which a single LG impulse drove f5 on 20 of 26 trials. (C) Stimulation of the LG axon at 200 Hz with intracellularly injected current excites f5 (top trace, dots). LG impulses are recorded extracellularly on the bottom trace. (D) IPSPs in a tonic flexor muscle fibre produced by LG-activation of f5. A train of eight LG impulses at 200 Hz evoked four f5 impulses (dots). Two EPSPs correlated with spikes in f1 or f2 (triangles) were also recorded. In A and C, LG impulses are also recorded on the trace from the superficial branch of the 3rd root (R3s).

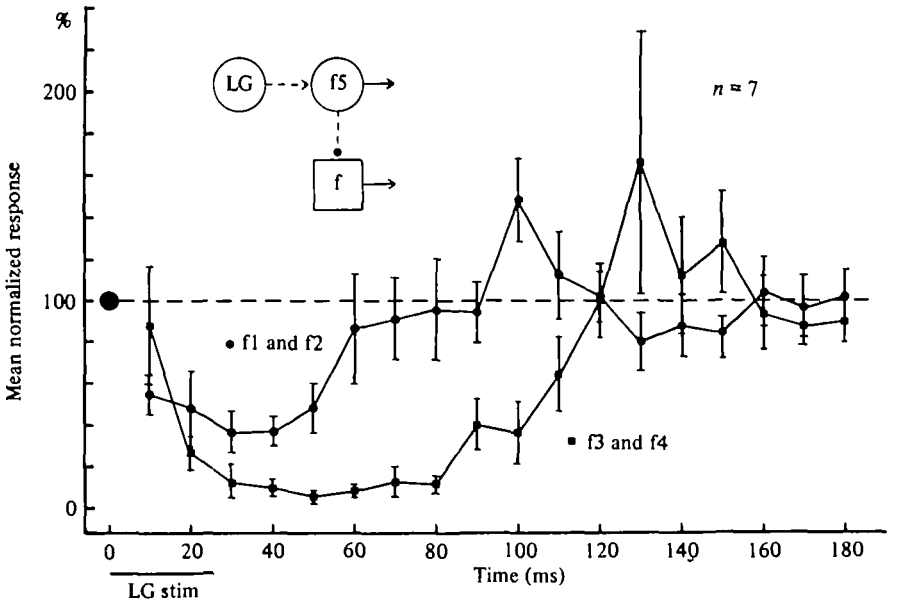


Fig. 4. LG-evoked inhibition of spontaneous activity in the tonic flexor motor neurones, followed by rebound. Inset shows the most parsimonious interpretation of the results. The graph was constructed as follows. For pairs of cells in each animal, a mean spontaneous spike rate per 10 ms was established on the basis of 200 ms samples taken between trials. For trials when the LGs were stimulated, the number of impulses in each bin on each trial was converted to a percentage of the mean spontaneous rate, and these were then averaged to give the bin scores for that animal. Each point on the graph is the mean of seven such scores from six different animals. Usually, recording was made from either ganglion 3 or 4. Each animal's score is based on a minimum of 27 or maximum of 46 trials. Bars indicate standard errors.

preparation studied, although the strength of the effect varied (Fig. 2). Even when excitation was strongest, the latency to an f5 impulse was too long to be consistent with an exclusively monosynaptic connexion (Fig. 3B).

To be certain that direct stimulation of an LG axon alone was sufficient to drive f5, we impaled an LG axon with a microelectrode and stimulated it with intracellularly injected current. The peripheral inhibitor was still excited (Fig. 3C), just as with extracellular stimulation.

Identification of f5 was usually based on its spike size, rate of activity and reciprocal firing pattern relative to the tonic flexor motor neurones, but positive identification was obtained in two preparations by making intracellular recordings from the slow muscle fibres concomitant with the extracellular recordings from the nerve (Fig. 3D).

*LG impulses inhibit spontaneous activity of the tonic motor neurones*

Tatton & Sokolove (1975) demonstrated that f5 inhibited the tonic flexor motor neurones, so it is not surprising that LG impulses, producing f5 activity, inhibit these motor neurones (Fig. 4). Using the same stimulation and recording procedure, inhibition was seen for all motor neurones except f6, which was not spontaneously active. The pair of smaller neurones (f1 and f2) was more weakly inhibited than the pair of larger cells (f3 and f4), but within each pair the cells were inhibited to the same degree. Therefore, in Fig. 4 the data for f1 + f2 are combined and plotted separately

from the combined data for f3 + f4. As shown in the inset, the inhibitory link between f5 and the tonic flexors is the most parsimonious way to explain the results. However, it will be shown in a later section that an additional inhibitory pathway is present.

*Tonic efferents 'rebound' from excitation or inhibition.* A delayed period of increased motor neurone activity follows inhibition (Fig. 4). Delayed excitation could result either from excitation arriving via a slow, polysynaptic pathway, or from postinhibitory rebound (see Perkel & Mulloney, 1974, Ref. 6). A delayed period of increased activity is not evident when the motor neurones are inhibited via antidromic stimulation of f5 (Tatton & Sokolove, 1975), which would seem to rule out inhibitory rebound with such stimulation. However, we used orthodromic stimulation which elicited bursts of f5 impulses and activated an additional inhibitory pathway (see below), thus producing more profound inhibition. Hence the experiments are not comparable and the possibility of inhibitory rebound cannot be dismissed. Delayed excitation following inhibition may have been what Roberts (1968*b*) observed in his experiments. The inhibition itself would have been easy to miss if the background rate were low. However, it is also possible that early inhibition and late excitation are separable events.

The peripheral inhibitor (f5) shows a period of *decreased* activity for 60–100 ms after being excited by the LGs (Fig. 3); a period of decreased activity in f5 also occurs when it is stimulated antidromically (Sokolove & Tatton, 1975). The tonic flexor motor neurones also show decreased firing after being stimulated antidromically (Sokolove & Tatton, 1975). In general, then, the tonic discharge rate of the flexor efferents is 'elastic' and rebounds from either excitation or inhibition.

*Effects of prolonged trains of LG impulses.* Although in intact animals the LG axons normally fire only single impulses or brief bursts (Wine & Krasne, 1972), we tested the effects of prolonged trains of LG impulses on the tonic flexor system, since prolonged trains have frequently been used to study the effects of single interneurones in the crayfish cord (e.g. Wiersma & Ikeda, 1964; Kennedy, Evoy & Hanawalt, 1966; Larimer & Kennedy, 1969). When trains of impulses were elicited in the LG axons, f5 was excited throughout the cord and the tonic flexor motor neurones were almost completed inhibited. This is illustrated in Fig. 5 A which shows a continuous recording from a tonic flexor root in response to a 2·2 s long, 100 Hz LG train. Following stimulation, the tonic motor neurones remained silent for about 0·5 s and then resumed firing at almost twice the rate they showed before stimulation. We also stimulated the LG axons with repetitive bursts at interburst frequencies of 10 Hz, which is within the range of tailflip frequencies seen during swimming (Fig. 5 B). This regimen also excited f5 and completely inhibited the tonic motor neurones. Since available evidence suggests that both giant and non-giant escape commands activate similar inhibitory pathways (Wine, 1977*a*; Krasne *et al.* 1977), the experiment shown in Fig. 5 B suggests that the tonic flexors will be inhibited throughout an escape swimming sequence.

*Inhibition of tonic flexor motor neurones without activation of the peripheral inhibitor*

Is the pathway involving f5 the exclusive inhibitory pathway between the giant axons and the tonic flexor motor neurones? To find out, we took advantage of the weak excitation of f5 by the giant axons in our experiment and observed the effects of LG bursts that were just short enough to be subthreshold for elicitation of f5 impulses.

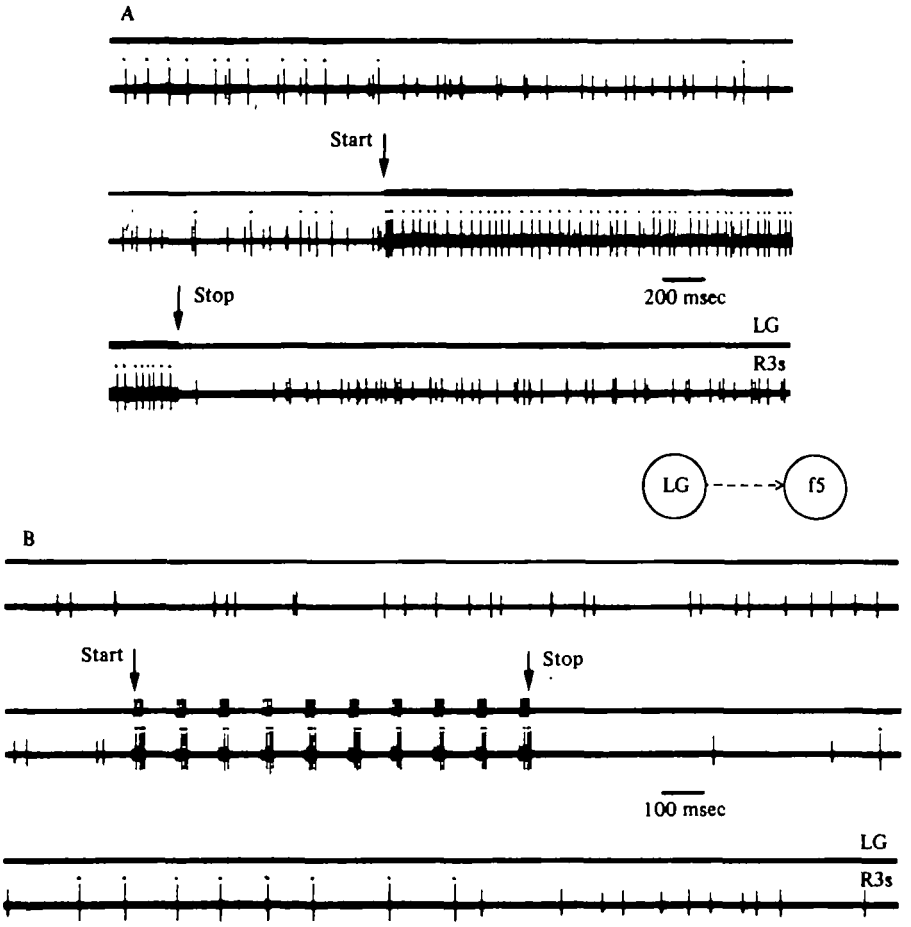


Fig. 5. (A) Effects of LG trains and repeated bursts. LG impulses at 100 Hz recruit f5 and suppress the tonic flexor motor neurones throughout the 2·2-second train. (B) Repetitive bursts of five LG impulses (impulse frequency – 200 Hz) at interburst frequency of 10 Hz also recruit f5 and suppress tonic flexor motor neurones. The records in A and B each are continuous. The top and bottom traces are simultaneous records showing the LG impulses and tonic flexor efferents, respectively. The LG impulses also appear on the tonic flexor record.

In these experiments we monitored both sides of the ganglion, to be sure that f5 was not activated on either side.

The results show that activation of f5 is *not* necessary for central inhibition of the motor neurones by giant axon impulses (Fig. 6). The histograms for f3 and f4 (Fig. 6A) show strong inhibition, but it is somewhat briefer than usual. Fig. 6B shows that f5 was not excited in most trials, although a slight increment above baseline can be detected when the responses are summed over 90 trials. Thus, the giant axons inhibit the tonic flexor motor neurones via two pathways, one involving f5, the other not.

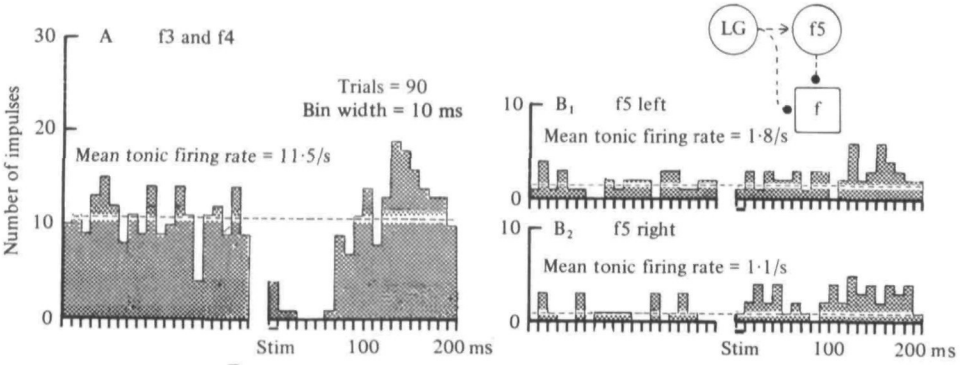


Fig. 6. Inhibition of tonic flexor motor neurones without activation of the peripheral inhibitor (f5). The LG train length was adjusted to be just subthreshold for f5 on most trials. Dashed lines show mean number of spontaneous impulses per 10-millisecond bin over all trials. Both sides were monitored during the experiment with equivalent results. (A) Inhibition of f3 and f4 on right side of ganglion 4. (B) Lack of early response in f5 on the left and right sides of ganglion 4. A slight increase in the firing rate of f5 is produced by the stimulus, but compare with Figs. 2 and 7B.

### *Inhibition produced by interganglionic, corollary discharge interneurones*

An impulse in any one of the four giant axons recruits a large number of interganglionic interneurones, so that both a burst of impulses ascending the cord and a descending burst can be recorded in any connective of the isolated cord for approximately 50 ms after the giant axon impulse (Wine, 1971). These 'corollary discharge' interneurones presumably serve many functions, including mediation of the extensive inhibitory effects observed *within* the escape circuitry (Roberts, 1968*a*; Kennedy, Calabrese & Wine, 1974; Krasne & Bryan, 1973; Wine & Mistick, 1977; Wine, 1977*a*, *b*). It therefore seemed important to determine if corollary discharge interneurones could also mediate inhibition of the tonic motor system.

The paradigm consists of stimulating one section of a transected medial giant (MG) axon while recording tonic motor activity in a ganglion on the other side of the transection (see Methods, and the inset in Fig. 7). Any effect seen on the other side of the cut axon must then be mediated by interganglionic interneurones that were recruited by the MG impulse before it reached the cut.

Impulses in a transected MG axon drove f5 and inhibited the tonic flexor motor neurones in a ganglion on the other side of the transection (Fig. 7), showing that corollary discharge interneurones are sufficient to mediate inhibition. Inhibition occurred both caudal to the block when the rostral portion of an MG axon was stimulated (data not shown), and rostral to the block when the caudal portion was stimulated. We did not test for inhibition in ganglia several segments away from the block, so we cannot judge the length of the relevant corollary discharge interneurones. Also, we did not attempt to see if inhibition could still be obtained in this situation without recruiting f5.

### *Tonic flexor reflexes*

A crayfish frequently responds to light touch by flexing its abdomen. Flexor tone also increased when isolated abdomens are touched (unpublished observations). In

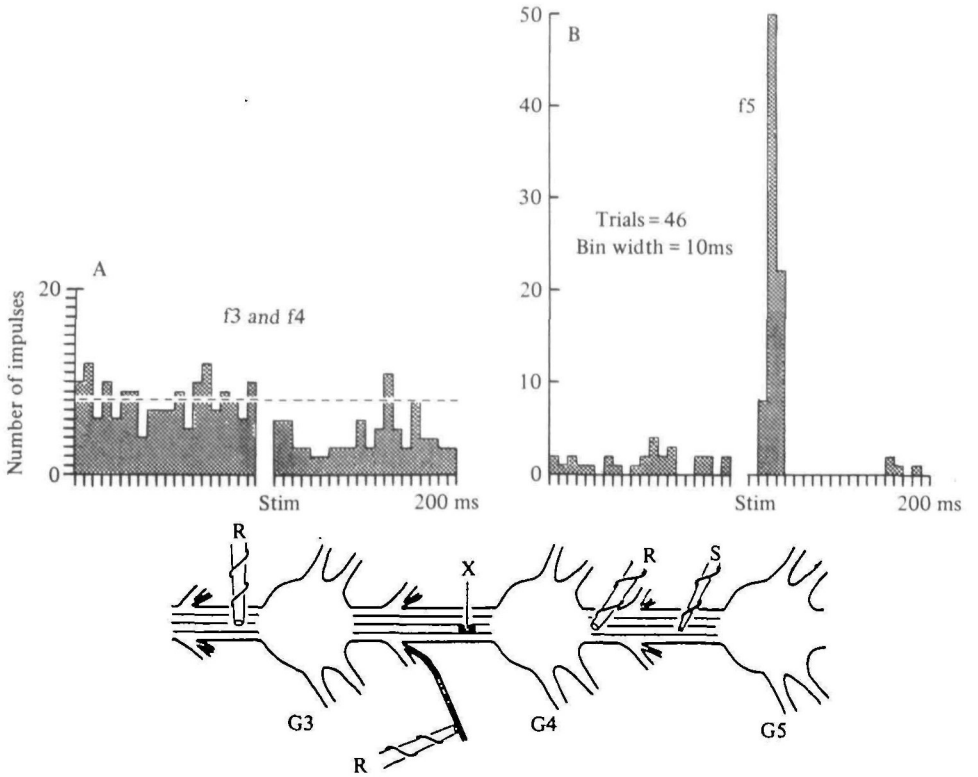


Fig. 7. Activation of f5 and inhibition of motor neurones by corollary discharge interneurones. Inset at bottom shows the experimental arrangement. One MG is disrupted between $G_3$ and $G_4$. (A) Stimulation of the blocked MG produces inhibition of f3 and f4 on the other side of the block. (B) Strong driving of f5 by stimulation of the blocked MG. Dashed lines show average number of impulses per 10-millisecond bin over the 46 trials.

isolated cords, an electrophysiological analogue of the postural response can be produced in flexor motoneurones by electrical stimulation of segmental sensory nerves. We quantified the response in order to investigate interactions of escape commands with tonic flexor reflexes.

The reflex response to stimulation of a sensory root is primarily the result of increased firing in f3 and f4 (Fig. 8A, B), the response in f1 and f2 being weaker, later, and more variable (Fig. 8C, D). The reflex can be obtained by stimulating any abdominal sensory root, but in our experiments the stimulus was always delivered to a 2nd root caudal to the root being recorded, and was limited to ganglia 2 through 4. The stimulation did not drive the inhibitor, f5, and motor neurone f6 was recruited in only one preparation.

### *LG inhibition of tonic flexor reflexes*

When stimulation of the sensory root was preceded by a brief train of LG impulses, the evoked response in f3 and f4 was reduced by about 55% (Figs. 9, 10). The following experiments indicate that this inhibition is not merely a trivial consequence of the previously demonstrated pathways that inhibit tonic activity, and that neurones afferent to the flexor motor neurones must also be inhibited by the LG impulses.

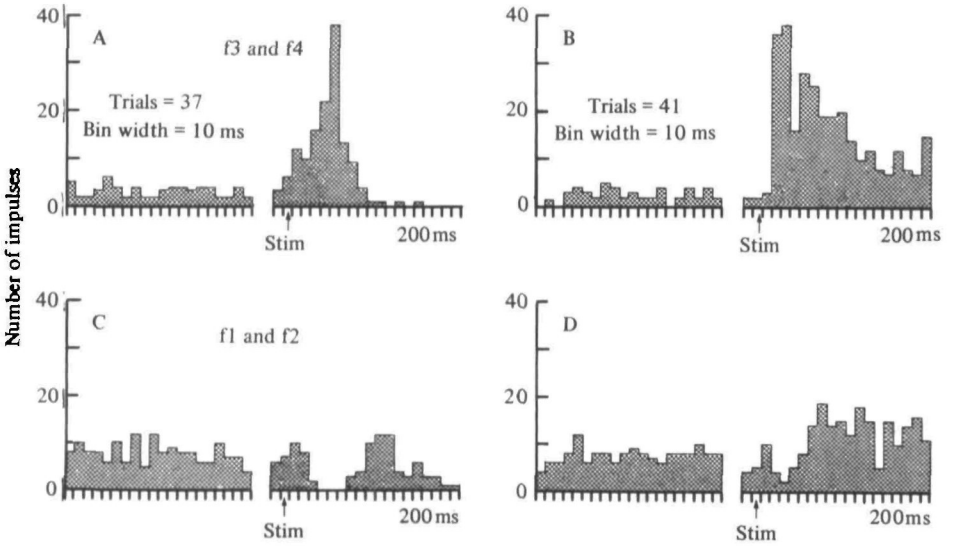


Fig. 8. Tonic flexor reflexes. (A) Excitation of f3 and f4 in ganglion 3 by stimulation of the ipsilateral 2nd root in ganglion 5. (B) In a different preparation, record from ganglion 4 while stimulating Root 2 of ganglion 5. (C) An unusual example of reflex inhibition of f1 and f2 recorded in ganglion 3 while stimulating the ipsilateral Root 2 of ganglion 5. (D) A typical result: weak reflex excitation of f1 and f2 recorded in ganglion 4, in response to stimulation of ipsilateral Root 2 in ganglion 5. Histograms similar to those in A and B were seen in every one of six animals tested.

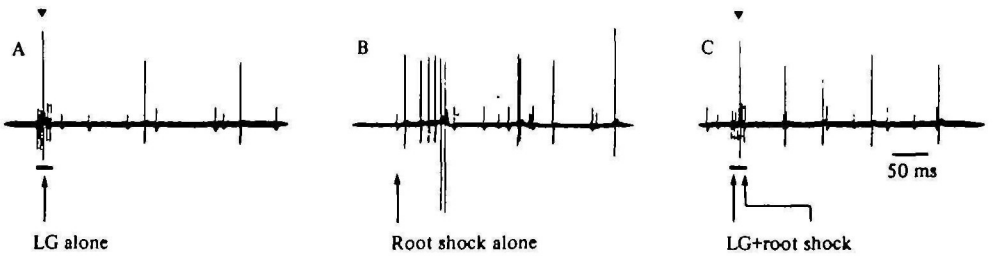


Fig. 9. Examples of inhibition of tonic flexor reflexes by LG impulses. Records from ganglion 3 in response to stimulation of the ipsilateral Root 2 of ganglion 5. Five LG impulses at 200 Hz were directly evoked in A and C.

### *Depression of tonic flexor reflexes during repetitive stimulation, and protection against depression by inhibition*

The following experiments were designed to demonstrate three points: (1) postural reflexes are depressed by repeated stimulation; (2) depression is site-specific, and is therefore likely to be the result of synaptic depression of the primary afferents; (3) depression is prevented by LG-evoked inhibition. All of these effects have previously been demonstrated within the LG escape system (Zucker, 1972*b*; Krasne & Bryan, 1973), and we shall argue that the parallels between the escape and postural systems suggest that some afferent pathways are shared between them.

*Depression and site-specific depression.* When repeated stimulation causes a reflex to

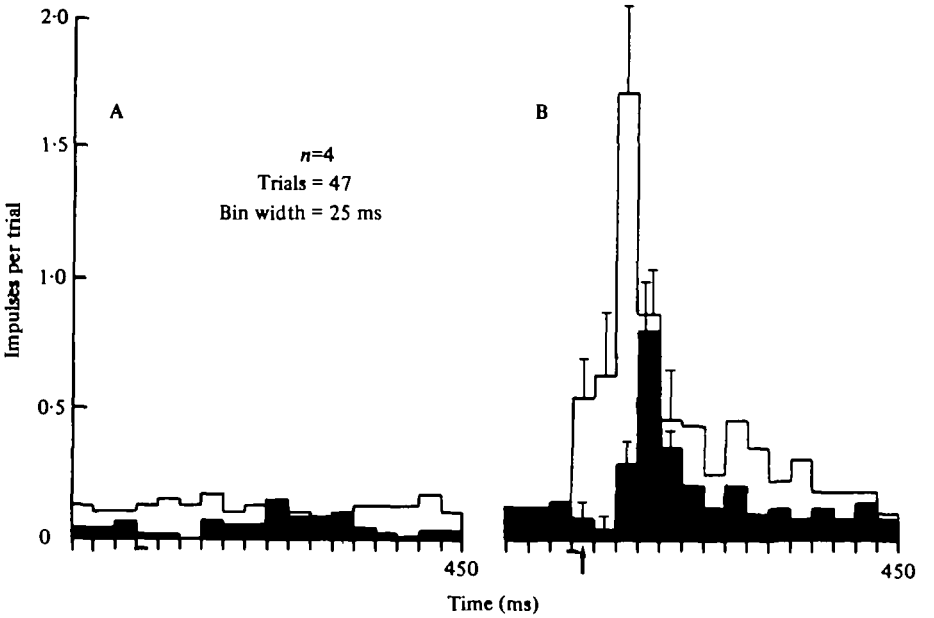


Fig. 10. Tonic flexor reflexes, and inhibition of reflexes by LG impulses. Scores are averaged for four preparations from 47 trials like the one shown in Fig. 9. (A) Spontaneous activity (light histogram) and inhibition by LGs (dark histogram). LG stimulus is indicated by bar overlapping the third and fourth bins. (B) Light histogram shows excitation of f3 and f4 by a stimulus to the 2nd root (arrow). Dark histogram shows inhibition of the response by prior LG activity (bar). Standard errors are shown for selected bins.

wane, transmission is decreasing somewhere in the pathway from receptors to effectors. Because reflexes almost always are evoked by highly convergent sensory input, transmission decrements that occur early in the reflex pathway will result in depression that is restricted to narrow sensory fields, while decrements at later stages will give rise to generalized depression. For example, repeated stimulation of a sensory root at frequencies above 0·2 Hz caused the reflex discharge of tonic flexor motor neurones to decline (Figs. 11–13). If repeated stimulation led to depression as a result of accumulating refractoriness of the motor neurone, we would expect to see complete generalization of depression: that is, responses to all sensory roots would be depressed no matter which one had been stimulated originally. Conversely, if reflex waning were due solely to depression of the synapses made by receptors, then no generalization would be observed: one set of receptors could be stimulated until the reflex was completely depressed, and then stimulation of neighbouring receptors would evoke a full response.

Our results show that depression of tonic flexor reflexes is site-specific. Two kinds of experiments were run to test for generalization. In the first experiment, we stimulated a 2nd root with three shocks spaced at 4-second intervals and recorded the decline in the reflex discharge of f3 and f4 in two different ganglia. We then repeated this test just 4 seconds after the 3rd of three shocks to a contralateral 2nd root, and found no sign of generalization whatsoever, although significant depression of the reflex occurred during each set of shocks (data not shown).

A more rigorous test for generalization was obtained by comparing the responses of tonic flexors to stimulation of each half of a 2nd root that had been divided surgically

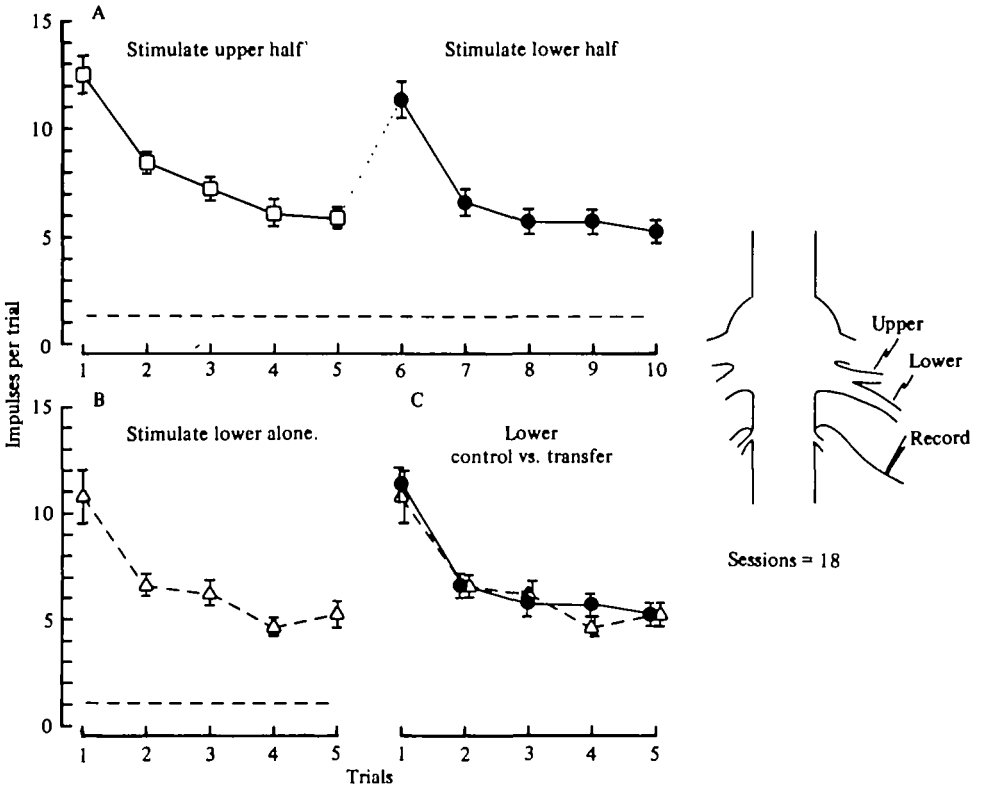


Fig. 11. Site-specific depression of tonic flexor reflexes. Inset shows the experimental arrangement. The tonic flexor root of ganglion 2 was monitored, while the split halves of the ipsilateral Root 2 of the same ganglion were stimulated. Each point on the graphs represents the average number of impulses recorded in f3 and f4 during a 500-millisecond period immediately following a 0·1-millisecond shock to one or the other half of the root. Eighteen sessions were averaged for each point. Dashed line indicates the average level of spontaneous activity for f3 and f4, measured as impulses/500 msec. (A) Responses to five stimuli to the upper half root followed by five stimuli to the lower half root, all at 3-second intervals. (B) Stimulation of lower half root alone. (C) Comparison of responses to stimulation of lower half root under control (triangles) or generalization (solid points) conditions. Bars indicate standard errors.

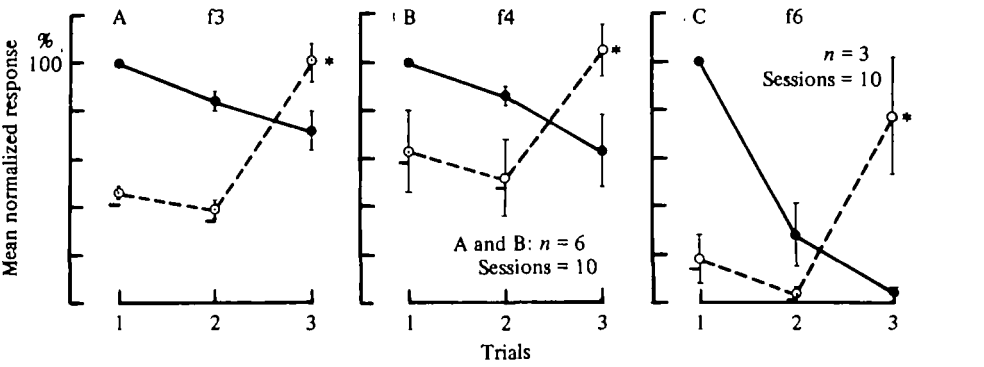


Fig. 12. Depression of tonic reflexes is prevented by LG impulses that precede the root shock. Each graph shows the mean, normalized responses to a shock to Root 2 for an identified motor neurone. Results from six different preparations were averaged for A and B, three preparations for C. Solid lines show depression to three consecutive shocks at 10-second intervals, dashed lines show 'protection' produced by stimulating the LG axons (bars) just before the root shocks on the 1st and 2nd trials. The 3rd trial in each condition consists of only the root shock. Asterisks indicate a significant difference between the responses on the 3rd trial. Bars indicate standard errors. See text.

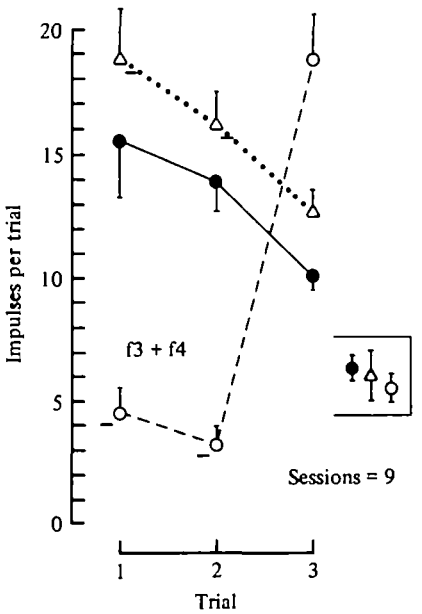


Fig. 13. Protection cannot be explained by sensitization. The depression caused by repetitive root shocks (solid line) is prevented by prior activation of the LGs (dashed line) but not by LG trains that follow the root shocks (dotted lines). Spontaneous firing rates for $f_3$ and $f_4$ were monitored between sessions during each condition and are indicated in the inset. Bars indicate standard errors.

(Fig. 11). In this experiment reflex responsiveness was tested by five shocks delivered at 3-second intervals to one of the halves of the root. In the test for generalization of depression, five stimuli were first delivered to the upper half root, then the sixth through tenth stimuli, still at 3-second intervals, were delivered to the lower half root (Fig. 11 A). As a control, the lower half of the root was stimulated alone, after at least a two-minute rest (Fig. 11 B). The entire regimen of test and control sessions was repeated 18 times. Fig. 11 shows that each of 5 stimuli caused significant depression of the reflex (first vs. last response: $P < 0{\cdot}001$). But, when the test (Fig. 11 A, solid points) and control (Fig. 11 B) responses are plotted together (Fig. 11 C) the curves are seen to be superimposable. Thus, prior depression of the reflex by stimulating one half root has no effect on the reflex caused by stimulating the other half root. A comparison of receptive fields of afferents in the root (Wine & Hagiwara, 1977) and of receptive fields of sensory interneurones (e.g. Wiersma & Hughes, 1961) makes it almost certain that afferents in the two halves of the root converge on many of the same sensory interneurones. Thus, the lack of generalization strongly suggests that the site of reflex depression is the first central synapse made by the afferents.

*Depression and protection.* In these experiments we compared the amount of depression obtained by repeated activation of the postural reflexes with the depression seen when reflexes were inhibited by prior activation of the LG axons. The logic of this paradigm, which was first introduced by Krasne & Bryan (1973; see also Bryan & Krasne, 1977*a*, *b*), is that inhibition will diminish depression if it is directed at a site prior to any depression-prone sites in the response pathway.

A trial consisted of either a single shock to a sensory root, or a 20 to 30-millisecond

train of 5 LG impulses immediately followed by a shock to a sensory root. 'Habituation' sessions comprised shocks to the root at 10-second intervals; 'protection' sessions were identical except that on the first two trials the shocks to the roots were preceded by five LG impulses. Each of six animals was run through 20 sessions separated by 5-minute intervals. Half the sessions were habituation and half protection, given in random order. For all conditions, the flexor motor response was defined as the total number of impulses in the 500-millisecond period immediately following the stimulus. We stimulated either a 2nd root (which is mainly sensory) or a 4th root of the last abdominal ganglion, which is sensory except for a single efferent.

The results are presented separately for flexor motor neurones f3, f4, and f6 in Fig. 12. Repetitive stimulation of a sensory root caused rapid depression of the evoked motor neurone response (Fig. 12A–C, solid lines); the third response was significantly depressed relative to the first (one-tailed t-test for differences between first and third responses: f3, $P < 0{\cdot}01$; f4, $P < 0{\cdot}025$; f6, $P < 0{\cdot}01$). Motor neurones f1 and f2 did not show significant reflex activation and so could not be tested for depression.

Depression was prevented for motor neurones f3, f4, and f6 by the preceding trains of LG impulses (Fig. 12, dashed lines). The difference between the last trial on protection and control runs was significant for all cells ($P < 0{\cdot}025$). When considered with our results on site-specific depression, the protection effect strongly suggests that LG-evoked inhibition is acting presynaptically on the terminals of afferents involved in postural reflexes.

*Control for sensitization.* An alternative explanation for the above results is that the LG stimulus might act to sensitize or dishabituate the reflex. A control experiment for a single animal is shown in Fig. 13. Habituation and protection runs are as before, but a control condition was added in which the first two stimuli to sensory roots were *followed*, 1 s later, by a train of 5 LG impulses (triangles and dotted line). The main finding is that the protection effect cannot be explained as dishabituation: the response on the third trial following two on which LG impulses *preceded* the root shock (dashed line) is significantly elevated relative to the response on the third trial following two on which LG impulses came *after* the root shock (dotted line).

LG trains do enhance the reflex, as shown by the upward shift of the response line. We have not examined this effect further, but the data show that sensitization is specific to the reflex – there is no increase in spontaneous activity caused by escape commands (Fig. 13, inset).

### *Summary of results: pathways for interactions*

The postulated inhibitory pathways from the escape command neurones to the tonic flexor system are shown in Fig. 14. Solid lines indicate monosynaptic connexions and dashed lines indicate functionally defined pathways. Pathway 1 is based on data in Figs. 2, 3, 4, and 7; Pathway 2 is based on data in Fig. 6; and Pathway 3 on results by Tatton & Sokolove (1975, their Fig. 4A). These are minimal pathways.

Tonic reflexes and interactions of escape commands with them can be explained most parsimoniously by assuming that the same mechano-sensory system which excites the LGs (Zucker, Kennedy & Selverston, 1971; Zucker, 1972*a*) also excites the tonic flexor motor neurones. There is no direct evidence that the same afferents go to both systems, but all indirect evidence is consistent with the hypothesis. The parallels

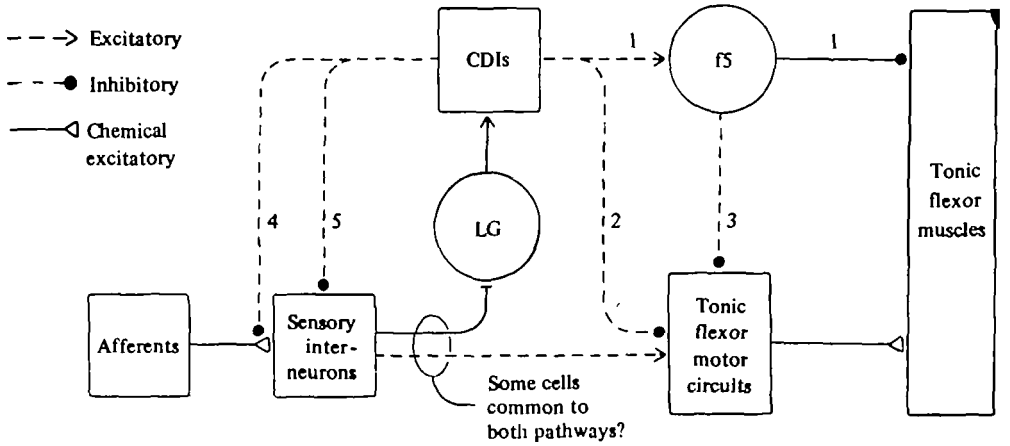


Fig. 14. Summary diagram. Relationship between LG command axons and tonic flexor system. Pathways 1 and 2 were established in this study; Pathway 3 was established by Tatton & Sokolove (1975). Afferents and sensory interneurones presynaptic to LGs are inferred to activate the tonic flexor motor circuits as well. Inhibition of tonic flexor reflexes occurs via Pathways 4 and 5 (Krasne & Bryan, 1973; Kennedy *et al.* 1974), and protection against tonic flexor depression via Pathway 4. Solid lines are monosynaptic connexions, dashed lines are functionally defined effects.

between the two systems are: (*a*) both respond to stimuli such as water-borne vibrations that excite the pit hairs (Wiese, 1976); (*b*) both show low-frequency depression with repeated stimulation (for LGs see Krasne, 1969; Zucker, 1972*b*; Wine, Krasne & Chen, 1975); (*c*) for both, depression is site-specific (see Zucker, 1972*b*); (*d*) for both, protection against the depression that occurs with repetitive stimulation is afforded by prior activity in the LG axons (Krasne & Bryan, 1973; Wine *et al.* 1975). Finally, shared sensory input to phasic and tonic systems is not unprecedented, since it has recently been demonstrated that the muscle receptor organs or stretch receptors (Alexandrowicz, 1951; Wiersma, Furshpan & Florey, 1953) project to phasic as well as tonic systems (Wine, 1977*c*). For these reasons, some of the sensory pathways to the LG are assumed to be shared with the tonic motor circuits, and Pathways 4 and 5 (Krasne & Bryan, 1973) become relevant to tonic reflexes.

Pathway 4 presynaptically inhibits the depression-prone chemical synapses between afferents and sensory interneurones (Krasne & Bryan, 1973; Kennedy *et al.* 1974) and prevents or diminishes depression. Pathway 5 (Krasne & Bryan, 1973) would enhance the inhibitory effect exerted against reflex activation of the tonics, but should not influence depression in this system. The implications of these pathways are considered below.

## DISCUSSION

Our chief findings are that the postural and escape systems interact in two ways: escape commands inhibit the postural system at all levels from afferents to muscles, and circumstantial evidence suggests that afferent circuits are at least partially shared. We did not test for reciprocal connexions from the postural system to the escape system.

### *Inhibition ensures functional independence*

It is surprising that the giant axons are the first identified interneurones shown to influence the slow flexor system. Although many axons can, when stripped from the connectives, drive or inhibit the slow flexors (Kennedy *et al.* 1966; Evoy & Kennedy, 1967), neither the structure of these cells nor the sensory pathways to them are known. Furthermore, they have usually been stimulated with long trains at high frequency to produce their effects. When the LGs are similarly stimulated, they also produce a clear-cut effect on the slow flexor system (Fig. 5A) and might be defined in terms of that system if their function as escape command cells were unknown.

Even short bursts of LG impulses inhibit the postural control systems, yet, as was mentioned earlier, the independence of the two neuromuscular systems has long been assumed. In fact, the two motor systems are relatively separate functionally, but this independence is not incidental – it is ***ensured*** by the extensive central interactions outlined above and previously (Wine, 1977*b*).

We have not performed any experiments to assess the functional significance of these inhibitory connexions, but we propose that by suppressing reflexes and tone in the postural system the animal increases the efficiency and precision of escape swimming. This proposal depends on the following observations and assumptions: (1) We assume that command-derived inhibition occurs during the repetitive tailflips of non-giant escape swimming, which often follows the initial, giant-mediated response (Wine & Krasne, 1972). Evidence supporting this proposal is that the fast flexor motor neurones, which are active during both giant- and non-giant-mediated tailflips, are the origin, via their connexions with corollary discharge interneurones, of at least some command-derived inhibition (Wine & Mistick, 1977; Wine, 1977*a*; Krasne, Wine & Kramer, 1977, and unpublished observations). (2) All available evidence (Evoy *et al.* 1967; Roberts, 1968*b*) suggests that the postural muscles are too slow to be used synergistically with the fast flexor muscles, and so would usually be out of phase with the fast flexors during swimming. (3) We assume that conflict between slow and fast muscles might introduce significant variability into the movements in spite of the disproportionality in muscle bulk. (In this regard, it should be pointed out that the fast *extensors* are not especially massive; furthermore, during many tailflips only the thinner, lateral divisions of the extensor muscles are active [J. J. Wine & G. Hagiwara, unpublished results].) If these arguments are correct, the escape systems should also inhibit the slow extensor system. Preliminary evidence suggests that they do (Wine, 1977*b*; van Lunteren, 1977).

### *Implications of multiple-level inhibition and shared afferents*

While the above reasoning offers a rationale for inhibition in general, it does not explain why inhibition should be directed at multiple levels of the postural system (Fig. 14). In considering this problem, we first note that no convincing function for peripheral inhibition in the postural system has ever been suggested, so that it is not profitable even to speculate on the significance of Pathway 1. However, we can suggest plausible functions for the remaining pathways. Inhibition of the motor circuits (Pathways 2 and 3) is necessary because the motor circuits are spontaneously active. Inhibition of afferent pathways to the postural motor circuits (Pathways 4 and 5) will,

as already indicated, selectively block reflexes. During escape, inhibition of sensory pathways simply reinforces the effect of inhibition at other levels, but it seems likely that under other circumstances Pathways 4 and 5 can be activated selectively, in order to block reflexes while permitting unimpeded activation of motor circuits by central commands.

An additional problem is raised by Pathway 4, which has the special property of protecting the sensory synapses against use-induced depression (Krasne & Bryan, 1973; Bryan & Krasne, 1977*a*, *b*). Until now, the significance of both depression and protection of these synapses has been specifically related to habituation of escape behaviour (e.g. Wine, Krasne & Chen, 1975). However, the commonality of the sensory pathways and the consequent response generality of both habituation and protection cause us to view these phenomena from a new perspective. Habituation is usually used in reference to a specific response, such as escape responses in crayfish, gill withdrawal in *Aplysia*, or leg flexions in the cat. However, in all these systems the depression-prone synapses are either the afferent terminals or stages just beyond the terminals (see Kandel, 1976). Thus the changes are primarily sensory ones and will affect various types of behaviour to the extent that the sensory pathways are shared. The importance of this view is that the functional significance of both synaptic depression and afferent inhibition may elude us if we view them as response-specific.

### *General considerations*

As our knowledge of different neural networks increases, we can expect to find ever more complex interactions among them. The well-studied escape system is a useful focus for such studies, because its extensive motor involvement and great rapidity demand that it have many inhibitory pathways to ensure its priority. The high priority of escape behaviour is likely to be a general phenomenon: Davis, Mpitsos & Pinneo (1974) have shown that escape interrupts every other behaviour they looked at in the marine mollusc *Pleurobranchaea*, and widespread inhibitory effects have been shown to emanate from the swimming 'command neurones' in *Tritonia* (Getting, 1977; Taghert & Willows, 1978). However, a crayfish does not always escape if presented with an adequate stimulus (see Davis *et al.* 1974). In fact, the decision to escape is subject to powerful inhibition that sometimes makes it virtually impossible to evoke a tailflip (Krasne & Wine, 1975). Thus, the likelihood that a response will occur, and a response's ability to interrupt other types of behaviour if it does occur, are separate aspects of behavioural interactions.

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