Brain Research, 214 (1981) 89–100

THE CRAYFISH LATERAL GIANTS AS COMMAND NEURONS FOR ESCAPE BEHAVIOR

GENE C. OLSON and FRANKLIN B. KRASNE*

Dept. of Psychology and Brain Research Institute, University of California at Los Angeles, Los Angeles, Calif. 90024 (U.S.A.)

(Accepted October 30th, 1980)

Key words: command neurons — lateral giants — crayfish — tailflip — giant fibers — escape — decision neurons

SUMMARY

The lateral giants (LGs) of the crayfish have often been described as command neurons. Recently, questions have arisen as to the definition of command neurons and as to whether the LGs are necessary for tailflips. We find that in an isolated abdomen preparation the LGs are necessary; temporarily blocking the LGs by hyperpolarization eliminates the tailflip flexor muscle output normally elicited by stimulation of the sensory afferents. However this demonstration of the LGs' necessity does not establish the LGs as the decision point of the behavior, for the LGs might be driven by a large input from a preceding decision neuron. We have checked for such an input by plotting the size of the EPSP produced in the hyperpolarized LG by various stimulus levels near spike threshold. We find no evidence for a large driving input near spike threshold. We conclude that the LGs have most of the features of command neurons.

INTRODUCTION

The concept of the command neuron was introduced by Wiersma to describe single nerve fibers that could elicit coordinated behaviors in the absence of sensory feedback[12]. This concept has a widespread appeal (judging from the number of cells proposed as command neurons), but the term is often invoked in ways that seem to go well beyond Wiersma's original usage[1,7]. In an attempt to clarify the situation Kupfermann and Weiss[5] have recently proposed a more formal definition for command

---

* To whom correspondence should be addressed at: Department of Psychology, 405 Hilgard Avenue, University of California, Los Angeles, Los Angeles, Calif. 90024, U.S.A.

neurons. Describing such cells as 'the critical decision points in the generation of behavior', they propose an operational test to classify cells as command neurons: the neurons must be shown to be both necessary and sufficient for the generation of a behavioral response. As Kupfermann and Weiss point out, satisfaction of both of these criteria has not been demonstrated for any proposed command neuron.

The lateral giant neurons of the crayfish (LGs) are among the most widely accepted candidates for command neurons. It has long been known that stimulation of the LGs is sufficient to elicit tailflips[11]. More recently, it has been shown that there are several different types of tailflips, differing in form, latency and neural organization[6,8,13]. The LGs are always associated with short latency flips that propel the animal primarily upwards, and such flips are almost exclusively elicited by phasic mechanical stimulation of the abdomen. The very close correlation between short latency LG-type tailflips and LG spikes suggests, but does not prove, that the lateral giants are necessary for such tailflips.

Based on anatomical and electrophysiological data together with the above observations, the LG tailflip circuit is usually diagrammed as in Fig. 1A[4,14]. However, the existing data are not sufficient to rule out the possibility (Fig. 1B) that there might exist a system of smaller, less rapidly conducting neurons parallel to the LG system. The action of such a system would normally be masked by the very fast LGs, though this alternate system by itself might be sufficient to produce LG-type flips. The LGs, then, could serve primarily to increase conduction speed or to synchronize the various segments and not be essential for a tailflip. In order to determine

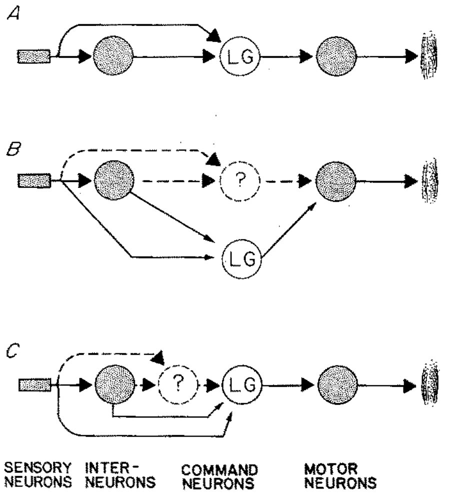


Fig. 1. A: the principle features of the LG escape reflex as generally diagrammed. Shading indicates that there are a number of such elements in parallel. Dashed lines represent conjectured circuitry. Although there are other pathways between the receptors and the tailflip motoneurons, it is assumed that the LG is the only path to the complete motor program. B: there could be an alternative system (dashed) that can evoke 'LG-like' tailflips. This path might be slower and/or might have a slightly higher threshold, so that it would normally be masked by the LG response. C: there could be a major 'decision' unit interposed between sensory elements and LGs; in this case the direct input of sensory elements to LG would be insufficient to fire the LG.

whether the LGs are indeed necessary we have examined the consequences of temporarily removing them from the circuit by hyperpolarization.

Even if it is shown that a neuron is both necessary and sufficient for the occurrence of a given behavior, this does not necessarily tell whether the neuron in question is really a 'decision point' for the behavior. For the possibility of a circuit such as that portrayed in Fig. 1C remains. In the present instance, there might exist an interneuron which receives most of the sensory input for the tailflip behavior and which has an overwhelming output to the LG. If the output of this cell is necessary and sufficient to fire the LG, then the LG may just serve to distribute the output of this trigger interneuron. Such a driver neuron might ordinarily go unnoticed if its input to the LG were to merge smoothly with the rising limb of the LG spike. We have checked for the existence of such a driving input by hyperpolarizing the LGs while examining the EPSPs produced by afferent inputs.

METHODS

Procambarus clarkii of both sexes measuring 6–8 cm from rostrum to telson were obtained from a local supplier. They were maintained in aerated, 10 gallon aquaria filled with dechlorinated water.

Experiments were carried out on isolated abdomens rather than whole animals, because rostral parts of the nervous system powerfully inhibit the LG reflex in animals that are restrained[3]. LG tailflips are normally elicited by phasic mechanical stimulation of the abdomen. To ensure repeatability of the stimulus and to allow fine control of stimulus intensity we used 0.10–0.25 msec electrical shocks applied directly to the tactile afferents (via 2nd abdominal roots) as stimuli, as in prior physiological work on this system[2,14]. To prevent dislodgement of the microelectrodes during tailflips, most of the flexor motor roots (third roots) of the abdomen were cut. Tailflips could be monitored by twitches of a few muscles that were left innervated and/or by monitoring electrical activity in the phasic branch of the 3rd roots.

Details of the dissection and recording instruments have been reported previously[2]. In brief, the abdomen was separated from the thorax after gradually cooling the animal to about 5 °C. The abdominal nerve cord was exposed by dissection of the terga and the overlying extensor musculature. All of the third roots except the right third root of the third ganglion were usually cut. Bipolar platinum hook electrodes were placed to stimulate the rostral end of the cord and the right second root of the third ganglion. Additional electrodes were used to record extracellular activity in the cord and in the intact third root of the third ganglion (Fig. 2).

The LGs are, in fact, chains of segmental neurons joined end to end by very effective electrical 'septal' junctions. They also communicate across the midline in each ganglion by electrical 'commissural' junctions[10] (Fig. 2). For recording PSPs produced by third ganglion, second root shocks, 2.5 M KCl-filled, 10–30 MΩ electrodes were placed at the caudal end of the LG segment that ascends from the 3rd ganglion; electrodes so positioned are fairly close to the LG dendrites of the 3rd ganglion (see Fig. 2). A second micropipette usually filled with 4 M potassium acetate was used to

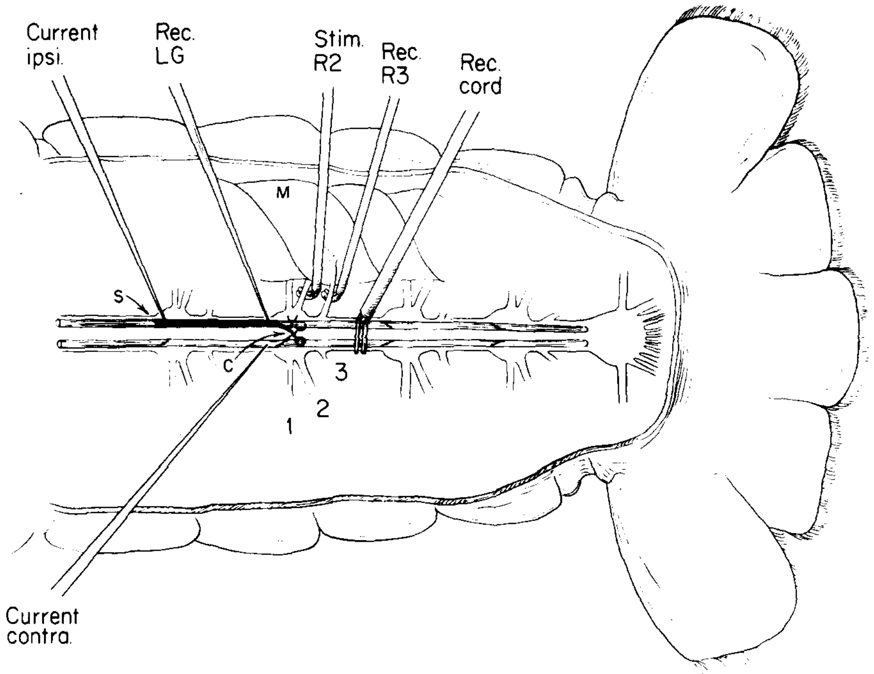


Fig. 2. Dorsal schematic view of the abdominal nerve cord in situ showing the lateral giant fibers and typical positions for stimulating and recording electrodes. S, septal; C, commissural synapse; M, flexor musculature; R2, 2nd root; R3, 3rd root.

apply the intracellular polarizing current. This second microelectrode was placed either in the LG on the same (right) side in the 2–3 connective, or in the contralateral (left) LG in the third ganglion (Fig. 2). These locations at some distance from recording electrodes were used for convenience. Current injected into the contralateral LG hyperpolarizes the ipsilateral LG via commissural connections. The intracellular polarizing current was derived from a Tektronix pulse generator isolated from ground by a radio frequency isolator. Current was limited by placing either a 50 or a 100 MΩ resistor in the circuit.

To determine if hyperpolarization blocked flexor muscle output, the second root was stimulated at various intensities at from 1 to 6 trials per minute. During these trials the LGs were alternately hyperpolarized or normal (unpolarized). When determining whether the LG is the decision point, the second root was stimulated at descending intensity levels at a rate of 4 per minute. Again alternate trials were hyperpolarized.

RESULTS

Lateral giants are necessary

As described by prior authors (for review see ref. 4), weak electrical shock of an abdominal second root elicits a compound PSP in the LG which grows as shock intensity is increased. Higher shock intensities often evoked twitches of the swimmerets (ventral abdominal appendages) without any obvious correlated change in LG intracellular response or any abdominal flexor activity. Finally, in good preparations there is a still higher, threshold level of stimulus intensity above which: (1) propagating spikes are produced in the LGs; (2) the swimmerets twitch, (3) spikes exit in 3rd motor roots of both sides of the 3rd and other ganglia, and (4) phasic flexor muscles that are still innervated contract.

Hyperpolarizing currents passed at either of the injection sites indicated in Fig. 2 hyperpolarize the LG at our 3rd ganglion recording site. The ipsilateral injection site (Fig. 2) gave roughly 0.1 mV/nA of polarization and the contralateral site about 0.01 mV/nA, consistent with previous reports[10]. Hyperpolarization has some effect on portions of the PSPs evoked by second root shocks. The PSP is normally multiphasic, consisting of a small, early, monosynaptic component (alpha), which is insufficient to evoke firing, followed by a potentially spike-producing disynaptic component (beta), and lastly by a somewhat variable and again smaller component (gamma) that is probably of mixed sign and origin[2,14] (and unpublished results). Hyperpolarization has no obvious effect on the alpha component. The rise and peak of the beta component were marginally affected, sometimes increasing and sometimes decreasing slightly. By contrast, hyperpolarization caused a reliable increase in the remainder of the PSP, which appeared as a slower decay of the PSP after the beta peak (see Fig. 4). These effects can be produced by current injected into either LG.

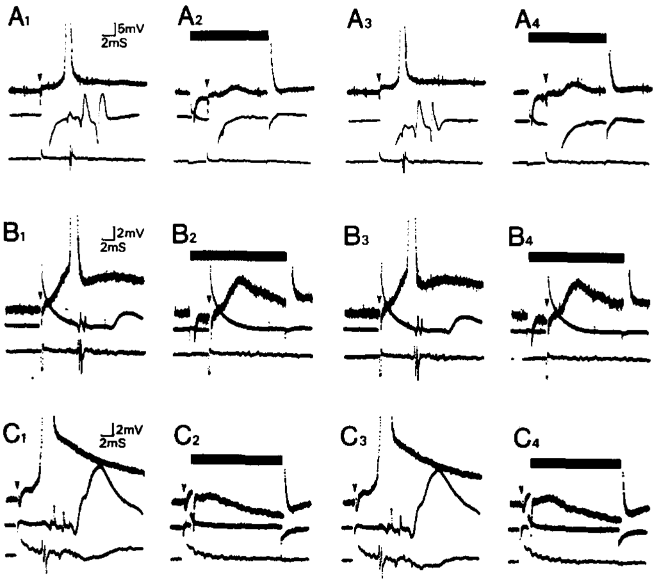


Fig. 3. Responses evoked by electrical stimulation of the second root. The 4 records in each row are a series of alternately normal and hyperpolarized trials. Each row is taken from a different preparation. In each figure the top trace is the intracellular recording in the LG; the middle trace is the extracellular recording from the third root; and the bottom trace is an extracellular recording from the dorsal surface of the cord in the 5–6 connective (to pick up propagated LG spikes). The heavy bar in columns 2 and 4 marks the duration of the hyperpolarizing pulse. The arrow marks the second root stimulation.

The effects of hyperpolarization on the LG reflex were unambiguous. Weak hyperpolarization caused no discernible effects on the 3rd root discharge or the flexor muscle twitch evoked by 2nd root stimulation, as long as the LG spiked. However, stronger hyperpolarization could block initiation of LG spikes to normally sufficient 2nd root stimuli and, when the LG spikes failed, the 3rd root discharge and the flexor muscle twitch were always eliminated. However, the swimmeret twitch often remained. Stronger afferent shocks could elicit LG spikes, 3rd root discharges, and flexor muscle twitches in a mildly hyperpolarized LG, and these could again be blocked by increasing hyperpolarization still further. The same effects are produced by current injected into either LG.

Fig. 3 illustrates these effects of hyperpolarizing the LGs. Each row is taken from a separate preparation and shows 4 trials at the same stimulus intensity. Columns 1 and 3 are the responses to 2nd root stimulation during normal (unpolarized) tests. The slow potentials in the third root records are muscle potentials of the intact flexor muscles. Note that the extracellular cord electrode shows that the LG spikes are propagating to other segments. Columns 2 and 4 are the responses to the same 2nd root stimuli when the LGs are hyperpolarized. The LG spike has failed in the 3rd ganglion, it is not propagated in the cord, and the 3rd root activity is absent.

Thus, the LGs appear to be necessary for a response. If a parallel system is present, it is not by itself sufficient to produce responses. It could be argued that a parallel system exists and, under normal circumstances, is sufficient to produce a response, but that when we hyperpolarize the LG the polarization spreads through electrical synapses to the parallel circuit or the motoneurons themselves and prevents the parallel circuit from producing a response. Were this the case, one would expect that when hyperpolarizing currents passed into the LGs are just barely sufficient to block the LGs, a self-sufficient parallel system should sometimes still be operative. However, when stimulus strength was fixed and hyperpolarization level varied continuously, we were never able to dissociate LG firing and motor response. Therefore, the parallel system hypothesis can stand only if it is assumed that hyperpolarizing currents passed into the LGs are more effective in blocking the parallel system than in blocking the LGs themselves. While this is conceivable, we think it more parsimonious to conclude that there is no sufficient parallel system.

The lateral giants as decision points

In the Introduction we raised the possibility that, though the LGs might be in the essential chain of command for LG escape and therefore be necessary and sufficient for production of behavior, a prior neuron might be the essential convergence point for sensory information and might determine the firing of the LGs. Were this the case, the subthreshold PSPs seen in LG when 2nd roots are shocked might merely serve to prime the LGs and bring them closer to critical firing level but not by themselves be able to fire the LGs, and there should be a large increase in the LG PSP whenever a stimulus exceeds the threshold needed for a tailflip and the hypothetical decision neuron is fired.

To uncover any such input we have examined the relationship between 2nd root

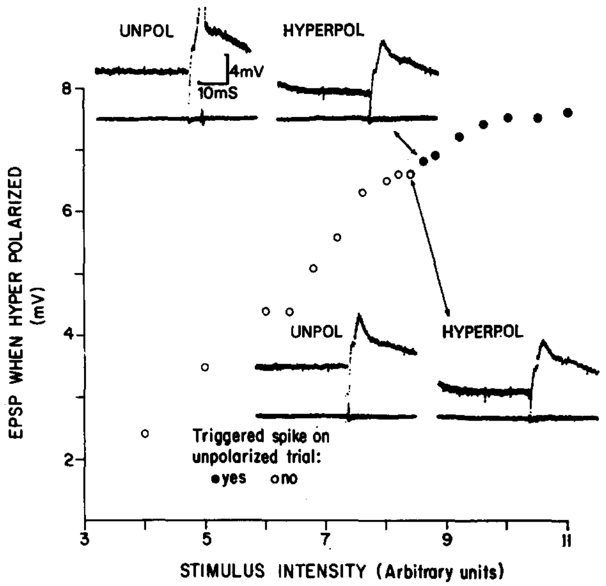


Fig. 4. The size of the PSP evoked in the hyperpolarized LG by 2nd root stimulation is plotted against the intensity of the 2nd root stimulus. Filled circles indicate that this stimulus level elicited an LG spike on the accompanying unpolarized trial. The insets are records of the LG PSP at the just-subthreshold and just-suprathreshold stimulus levels indicated.

stimulus strength and PSP size in hyperpolarized LGs. Each stimulus intensity level was used for two trials; one normal and one hyperpolarized. The normal trials were used to determine sensory threshold for LG tailflips; hyperpolarized trials were used to measure LG PSP magnitudes uncontaminated by spikes. The 2nd root was stimulated at descending levels of intensity to eliminate the problem of bringing in unhabituated

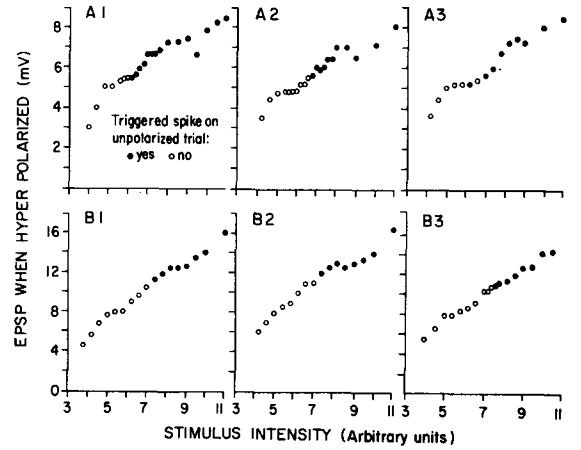


Fig. 5. These are graphs of additional trials of the threshold experiment of Fig. 4, but taken from two separate preparations. Row A was a preparation hyperpolarized by current injected into the contralateral LG. Row B shows the results of the same experiment but with current injected ipsilaterally.

afferents whose effects may quickly habituate, thus increasing variability. The results of such an experiment are shown in Fig. 4. The insets show the 4 consecutive intracellular recordings of the lateral giant just above and below spike threshold. The lack of a substantial jump in the hyperpolarized PSP size at spike threshold indicates that the lateral giant is not driven by a large input from a single trigger cell. Fig. 5 shows several additional trials of the same procedure in two different preparations, one which is hyperpolarized by current injected into the same cell (lateral giant), and one polarized by current injected into the contralateral LG. In no case is there evidence for a large ('decisive') input near the lateral giant threshold. Thus the lateral giant does appear to be the principle integration point for the decision to produce a tailflip.

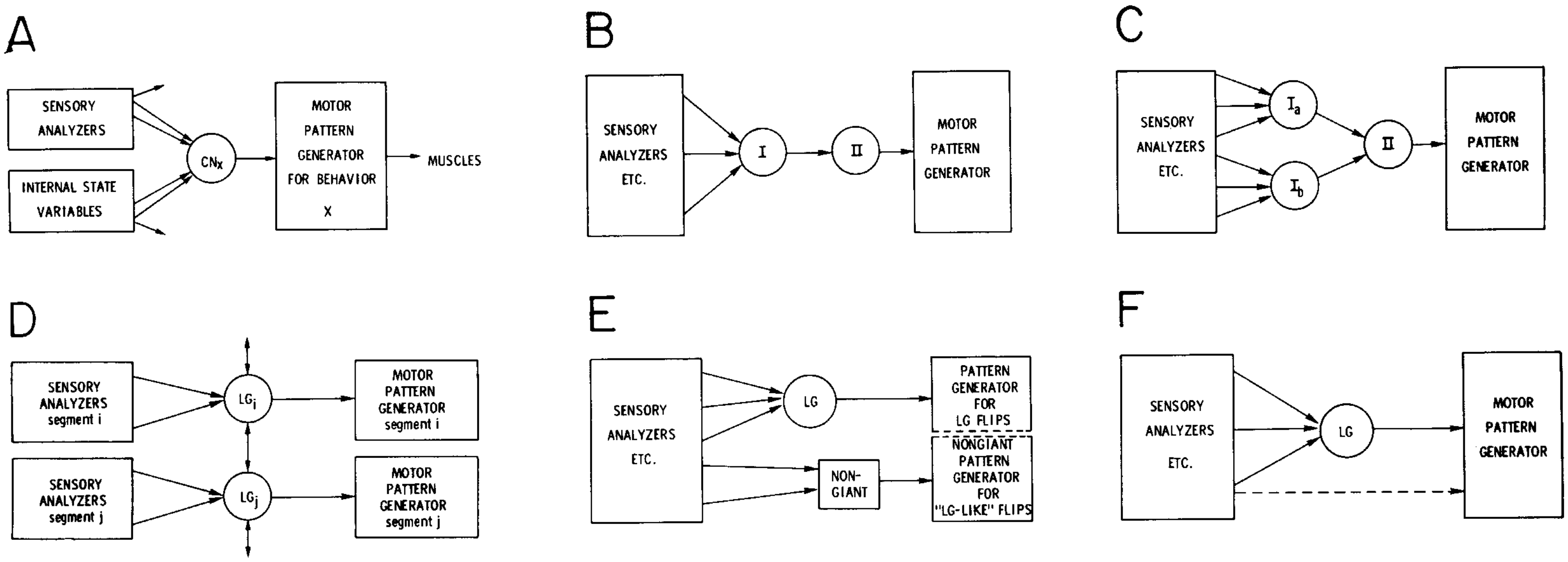
DISCUSSION

Based upon experiments on crayfish swimmeret movements and on the tailflip escape response that is under discussion here, C.A.G. Wiersma proposed that nervous systems contain individual 'command' or 'trigger' neurons that by themselves can drive complex, coordinated motor sequences. This idea has great appeal, because it brings to mind a picture (Fig. 6A) of the relationship between sensory inputs and motor outputs which greatly simplifies our conceptions about the organization of the nervous system. The simplicity results from the fact that the 'command neuron' in such a scheme is the single point of stimulus response interaction. It is at once a unique focal point on which selected sensory input, or perhaps the output from selected feature detectors, converges and a gateway or 'push button' for activating motor circuitry that generates the appropriate motor pattern (i.e. the motor pattern appropriate to those stimulus configurations which fire that particular command neuron). The question of whether command neurons of this sort really exist has never been adequately answered, even for the neurons that inspired Wiersma's original idea.

Based on the fact that the firing of a command neuron as in Fig. 6A would be both sufficient for producing the normal behavior pattern and prerequisite to the occurrence of the pattern to a natural stimulus, necessity and sufficiency have recently been suggested as an operational definition for command neurons[5]. Empirical demonstrations of such necessity and sufficiency have now been provided for the LG escape response. This is the first time that this has been done for any putative command neuron. Strictly speaking, our demonstration of necessity applies only for the stimuli

---

Fig. 6. A: in this conception CN_x is the sole gateway to the motor pattern generator for behavior X. Certain combinations of external stimuli and internal states produce enough converging input to fire CN_x. B: sensory system convergence occurs at I, 'decision level', whereas II, 'command level', drives the motor pattern generator. C: different 'decision neurons' (level I) are fired by different stimulus patterns. Input from a single decision neuron drives II. D: the LG system is a chain of coupled segmental command neurons. This arrangement combines levels I and II of Fig. 6C. E: certain stimuli can elicit non-giant tailflips that resemble LG flips but are not mediated by giant fibers. These two types of tailflips utilize many of the same muscles. The extent to which the pattern generators overlap is unknown. F: some sensory input bypasses the LG and reaches the flexor motor neurons directly. The effect of this input is unknown.


A
SENSORY ANALYZERS
INTERNAL STATE VARIABLES
CN_x
MOTOR PATTERN GENERATOR FOR BEHAVIOR X
MUSCLES
B
SENSORY ANALYZERS ETC.
I
II
MOTOR PATTERN GENERATOR
C
SENSORY ANALYZERS ETC.
I_a
I_b
II
MOTOR PATTERN GENERATOR
D
SENSORY ANALYZERS segment i
SENSORY ANALYZERS segment j
LG_i
LG_j
MOTOR PATTERN GENERATOR segment i
MOTOR PATTERN GENERATOR segment j
E
SENSORY ANALYZERS ETC.
LG
NON-GIANT
PATTERN GENERATOR FOR LG FLIPS
NONGIANT PATTERN GENERATOR FOR "LG-LIKE" FLIPS
F
SENSORY ANALYZERS ETC.
LG
MOTOR PATTERN GENERATOR

we used. However, while artificial out of experimental necessity, our root shock stimuli probably produced afferent volleys not too different from ones due to optimal natural stimuli, which are best when sudden and fairly wide-field.

The existence in a circuit of a neuron that passes the tests of necessity (for known stimulus situations) and of sufficiency, by no means proves that the circuit is organized exactly like Fig. 6A. For example. Fig. 6B and C diagram circuits in which functions we might call 'decision' (I) and 'command' (II) are separated. In Fig. 6B both level I and level II neurons would pass the tests of necessity and sufficiency; in Fig. 6C neurons of both levels would be sufficient and those of level II necessary. But in both cases the locus of sensory convergence is different than in Fig. 6A. The results illustrated in Figs. 4 and 5 (demonstrating the lack of a driving input) rule out such a conception in the present case, and are consistent with the simpler diagram of Fig. 6A so long as we regard the LGs as a single functional unit. However, if we consider the fact that the LGs are really chains of closely coupled but independent neurons, we must diagram the circuit in which they participate as in Fig. 6D; here each individual (segmental) LG neuron combines the function of decision and command when stimuli are applied to its own segment but functions as a driven relayer of a decision made elsewhere when stimuli are applied to other segments.

A special problem arises when one tries to test necessity due to the logical impossibility of ever really proving necessity. Among the infinity of imaginable stimulus situations and states of the animal it will always remain possible that eventually one will be found that can produce the behavior without the firing of our putative command neuron. In the present case we present evidence that removing the LG eliminates tailflips evoked by 2nd root stimulation in the isolated abdomen. However, we cannot rule out the possibility that similar (or the same) stimuli might evoke tailflips without the LGs in some conditions. In fact, it is already well known that tailflips very similar to those mediated by the LGs can occur without LG firing in response to gradually applied mechanical stimulation of the abdomen when the animals nervous system is intact[13]. Thus, Fig. 6F provides a more accurate portrayal of the organization of LG-like escape responses than does Fig. 6A.

The test of sufficiency raises other difficulties. It is easy to envisage circuit organizations that are very similar in spirit to that of Fig. 6 but that lack individual neurons that are sufficient to produce full behavior patterns. In particular, a pattern generator might have to be 'primed' by input directly from sensory analyzing circuitry (as in Fig. 6F) in order for the 'command' neuron to elicit behavior. In fact, although the LGs alone are sufficient to produce a fair approximation to normal behavior[11,13], Fig. 6F provides a more accurate picture of their connections than does Fig. 6A because fast flexor motor neurons do receive some sensory derived input that by-passes the LGs[9]. The effect of this input on the tailflip is not yet known.

Thus, the circuit in which the LGs are embedded approximates the organization of Fig. 6A but does not conform to it ideally. Insofar as it does conform, it seems appropriate to refer to LGs as the *command neurons* of the circuit.

We believe, however, that questions of whether particular neurons 'are' or 'are not' command neurons and of what the definition of such a neuron — operational or in terms of status in a circuit — ought to be, to some extent miss the most important point. What we actually want to know is not whether particular neurons should or should not be classified as command neurons but, rather, how various neural circuits are organized. Circuits such as Fig. 6, which have in them neurons that clearly serve command or triggering functions embody or, better, suggest a number of separable features that each simplify thinking about the nervous system. These circuit features include the following. (1) Single neurons can act as 'push buttons' which release entire motor patterns. (2) In extreme cases there may be associated with each motor pattern one and only one such neuron that is always the immediate cause of the pattern's occurrence no matter what stimulus events and individual state variables precipitate the response. (3) The motor pattern is produced by an entirely endogenous generator whose operation is: (a) independent of the particular stimulus events that initiated the pattern, and (b) independent of feedback during execution of the pattern. (4) There is a unique, well-defined decision point in the circuit mediating between a given stimulus and response pattern. (5) Sensory analyzing circuitry is separate from motor generation circuitry except at a single decision making level of integration, i.e. the command neuron. The sensory analyzing circuitry prior to this level is not response coded, i.e. there are not separate sets of sensory neurons dedicated to each response.

Probably none of these possible generalities holds perfectly even for the sort of escape responses investigated here, though most of them do hold to a first approximation. Experimentation aimed at assessing the extent to which any of these features hold for other behavior patterns having different functional requirements will be worth very much more than experiments designed to formally classify particular neurons as command neurons.

ACKNOWLEDGEMENTS

This work was supported by USPHS Grant NS8108. Gene C. Olson was an NIMH Trainee on Grants MH06666 and MH15345.

REFERENCES

1 Bowerman, R. F. and Larimer, J. L., Command fibers in circumoesophageal connectives of crayfish. 1. Tonic fibers, *J. exp. Biol.*, 60 (1974) 95–117.

2 Krasne, F. B., Excitation and habituation of the crayfish escape reflex: the depolarizing response in lateral giant fibers of the isolated abdomen, *J. exp. Biol.*, 50 (1969) 29–46.

3 Krasne, F. B. and Wine, J. J., Extrinsic modulation of crayfish escape behaviour, *J. exp. Biol.*, 63 (1975) 433–450.

4 Krasne, F. B., Wine, J. J. and Kramer, A. P., The control of crayfish escape behavior. In G. Hoyle (Ed.), *Identified Neurons and Behavior of Arthropods*, Plenum Press, New York, 1977, pp. 275–292.

5 Kupfermann, I. and Weiss, K. R., The command neuron concept, *Behav. Brain Sci.*, 1 (1978) 3–39.

6 Larimer, J. L., Eggleston, A. C., Masukawa, L. M. and Kennedy, D., The different connections and motor outputs of lateral and medial giant fibers in the crayfish, *J. exp. Biol.*, 54 (1971) 391–402.

7 Rosenbaum, D. A., Selective adaptation of 'command neurons' in the human motor system, *Neuropsychologia*, 15 (1977) 81–90.

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

9 Sherwood, D. N. and Wine, J. J., Orderly sequence of polysynaptic sensory inputs to crayfish tail-flip motoneurons, *Neurosci. Abstr.*, 5 (1979) 261.

10 Watnabe, A. and Grundfest, H., Impulse propagation at the septal and commissural junctions of crayfish lateral giant axons, *J. gen. Physiol.*, 45 (1961) 267–308.

11 Wiersma, C. A. G., Function of the giant fibers of the central nervous system of the crayfish, *Proc. Soc. exp. Biol. Med. N.Y.*, 38 (1938) 661–662.

12 Wiersma, C. A. G., The organization of the arthropod central nervous system, *Amer. Zoologist,* 2 (1962) 67–78.

13 Wine, J. J. and Krasne, F. B., The organization of escape behavior in crayfish, *J. exp. Biol.*, 56 (1972) 1–18.

14 Zucker, R. S., Crayfish escape behavior and central synapses. I. Neural circuit exciting lateral giant fiber, *J. Neurophysiol.*, 35 (1972) 599–620.