treated and mated to virgin females from the Raleigh stock (*4*). Fecundity of the resulting F_1 (virgin) females was very high. Lineage of each daughter tested was recorded to detect lethal clusters. The data in Table 1, Series B, have been corrected for the pair in which clustering was found. The data from these tests again indicate that both tepa and its nonalkylating analog, hempa, are mutagenic. Lethal frequencies of 31 and 61 times the control value resulted from 15- and 45-minute exposures, respectively, of males to hempa. Ten-minute exposures to a film of tepa induced 21 times more lethals than were present in the control. However, the 15-minute treatment yielded a lower frequency of recessive lethals. The longer treatments produce higher levels of dominant lethal mutations and significant amounts of sperm inactivation (*6*). Data from the tests support the following conclusion: both tepa and its nonalkylating analog, hempa, are mutagenic.

Tepa is about 100 times more effective than hempa in inducing recessive lethal mutations in *Bracon hebetor*. Other studies (unpublished), utilizing males treated simultaneously with the males used in the present experiments, showed that tepa is similarly more effective than hempa in producing sterility (dominant lethal mutations or sperm inactivation, or both).

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1. S. C. Chang, P. H. Terry, A. B. Borkovec, *Science* **144**, 57 (1964).
2. Tepa is tris(1-aziridinyl)phosphine oxide. Hempa is hexamethylphosphoric triamide.
3. A. R. Whiting, in *Advances in Genetics*, E. W. Caspari and J. M. Thoday, Eds. (Academic Press, New York, 1961), vol. 10, p. 295.
4. Fertilized eggs usually give rise to diploid females only, providing the parents come from unrelated stocks with different sex alleles. In crosses of closely related individuals, some fertilized eggs give rise to diploid males that are homozygous for the sex alleles. These males are highly inviable. For this reason we utilized males from a mutant stock (either No. 1 white-eyed or lemon body) crossed to wild-type females from stocks with different sex alleles (No. 33+ or Raleigh). These *Bracon* stocks are known by this nomenclature to geneticists familiar with this organism.
5. To determine the frequency of recessive lethal mutations, one collects virgin F_1 daughters from treated males crossed to untreated females. These virgin daughters are then allowed to lay eggs. If a female is free of recessive lethal factors (which are expressed in the egg stage), all her eggs should hatch. If she is heterozygous for one recessive lethal, 50 percent of her eggs should hatch; if she is heterozygous for two unlinked lethals, 25 percent should hatch; and so forth. Although the method does not exclude chromosomal rearrangements, this technique allows the investigator to detect recessive lethal factors on any of the 10 chromosomes contained in the sperm. Thus he is not limited to detecting only those recessive lethals located on the X chromosome or a single autosome.
6. L. E. LaChance *et al.*, unpublished.

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Release of Coordinated Behavior in Crayfish by Single Central Neurons

Abstract. *By stimulating and recording from the same interneuron at two separate points, we have shown that coordinated output to the postural abdominal muscles of crayfish can be produced by electrical stimulation of a single cell. Several central neurons can individually initiate one type of movement (for example, flexion), each producing a unique abdominal geometry.*

Complex, coordinated behavior can be evoked by electrical stimulation of certain brain regions in vertebrates (*1*) and invertebrates (*2*). Since these effects are produced with relatively gross electrodes, they undoubtedly depend upon the activation of large numbers of neurons by the stimulating current. In the nervous systems of some arthropods, however, activation of a very few cells may release similarly stereotyped motor patterns. For example, the abdominal appendages of the crayfish, the swimmerets, normally beat in a metachronous fashion (*3*); Wiersma and Ikeda (*4*) generated patterns of motor-nerve discharge appropriate for this behavior by stimulating fine bundles of fibers isolated consistently from the same region of the central nervous system.

Such experiments strongly suggest that ordered output can be released by activity in single interneurons. Working with the system controlling antagonistic postural muscles in the crayfish abdomen, we have tried to demonstrate rigorously that single-cell stimulation can produce such patterns, to find the sensory inputs for these cells, and to discover how several such elements producing similar actions differ from one another. On one side of an abdominal segment, the slow extensor and flexor muscles are each supplied with six efferent neurons; five of these are motor, and one is a peripheral inhibitor. These neurons have characteristic sizes and activity patterns and, therefore, can be individually identified in an electrical record from the appropriate nerve (*5*). There are a limited number of central elements (*6*) which, when stimulated, cause a coordinated motor output—that is, a set of central effects appropriate to either flexion or extension. The flexion command involves excitation of the five flexor motoneurons, inhibition of the peripheral inhibitor to the flexors, excitation of the extensor inhibitor, and inhibition of the five extensor motoneurons; extension command fibers mediate precisely the opposite actions. These effects are normally seen in several segments at once. If single cells are indeed responsible for the entire output, then it must be concluded that one interneuron can influence the discharge of at least 120 efferents.

The procedure used was similar to one developed for analyzing the point of impulse initiation in interneurons (*7*). Crayfish (*Procambarus clarkii*, collected locally) were pinned ventral side up in a chamber containing van Harreveld's solution (*8*). The nerve cord was exposed, and its sheath was removed between ganglia 1 and 2 (rostral site) and between 5 and 6 (caudal site). Bipolar recording electrodes were placed under the superficial third roots supplying the slow flexor muscles on the right side of segments 2, 3, and 4. The saline level was then lowered, oil was layered on top, and the recording electrodes carrying the nerves were lifted into the oil layer. Signals from these three recording sites, each monitoring the discharge of six efferent neurons, were amplified and displayed on a multichannel oscilloscope. Bundles of nerve fibers were stripped from the rostral site by fine dissection; they were left connected caudally and drawn over a pair of platinum wires in the oil layer for stimulation with brief (0.1 msec) pulses of current. Most bundles had no effect upon flexor motor discharge when stimulated, but some produced coordinated flexor or extensor output at a sharp and reproducible threshold intensity. If necessary, active bundles were further dissected until a strand was obtained that produced a pure effect. The pair of electrodes carrying the strand was then switched to an amplifier connected to a fourth oscilloscope trace. At this point, a similar dissection was begun in the homologous region of the caudal site, these bundles being left attached rostrally. In successful experiments, electrical stimulation of a series of such

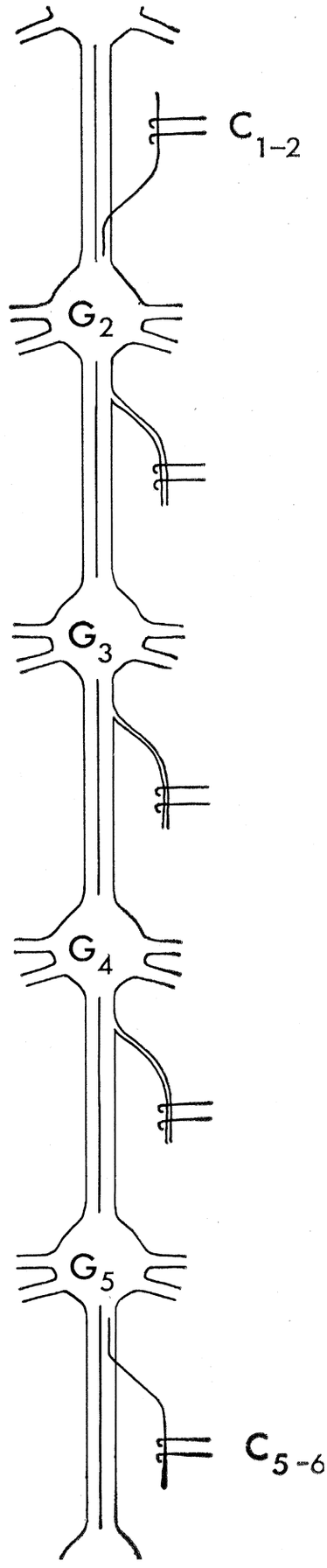

Fig. 1 (right). Diagram of the experimental arrangement. Two regions of the same interneuron are isolated in the vertical nerve cord, between ganglia 1 and 2 and between 5 and 6, with the intermediate portion intact; they may be either stimulated or recorded through the electrodes labeled C_{1-2} and C_{5-6}. The slow flexor motor output from ganglia 2, 3, and 4 is also recorded by electrodes placed on the superficial branches of the third roots.

bundles would eventually reveal one that produced a single all-or-none impulse in the record from the rostral filament. The preparation thus provided access to two separate points on the same central interneuron; the latter remained connected to three segmental output channels between these two stimulating or recording sites. Figure 1 is a diagram of the experimental arrangement.

We performed these tests to confirm that the motor effect was unambiguously the result of evoked activity in a single central neuron: (i) The caudal filament was stimulated at 100 times per second, and a train of constant-latency, all-or-none impulses was evoked in the rostral filament. (ii) An equivalent stimulus train was applied to the rostral filament, and a similar response was obtained from the caudal filament. (iii) The conduction velocity was calculated for both directions of propagation and proved to be identical. (iv) Motor effects were evoked at the same stimulus intensity required to produce impulses at the distant recording site. (v) Simultaneous stimulus trains were delivered to both ends and produced an effect upon motor output no greater than that resulting from activation of one end alone.

Figure 2 shows the effect characteristic of flexion command fibers. During stimulation (A and B), the discharge frequency of several motor axons in the third root increased. Stimulation at the rostral site (A) or at the caudal site (B) produced fixed-latency impulses at the other location. Figure 2, C and D, with an expanded time-base, illustrates these responses for single shocks. The intervals between stimulus and response are identical; the conduction velocity is 4.4 m/sec, a value which would suggest the fiber diameter is in the range of 20 μ, but our technique unquestionably selects for large axons. Figure 2E is a record taken from the rostrally isolated filament before caudal dissection; passive flexion of the ipsilateral uropod blades produced impulses in the fiber and simultaneously excited flexor motoneurons. Thus sensory inputs to the command fiber can be identified, and they have an action identical with that produced by electrical stimulation of the central neuron itself. A command fiber producing extension is shown for comparison in Fig. 3. The intensity of repetitive (100 per second) caudal stimuli was increased gradually, so that the rostral filament showed responses first to occasional and then to each stimulus pulse. The rostrally recorded impulses are all-or-none, and the motor effect develops at the same threshold as that for the conducted impulse in the central axon. In contrast to the effect of the flexion command unit in Fig. 2, the discharge of all excitatory axons is suppressed, and a previously silent efferent neuron (identified as the flexor inhibitor) is excited. Although records from the root supplying the slow extensor muscles are not shown here, other experiments (*4*, *6*) have demonstrated that central elements producing this pattern of flexor output always simultaneously excite extensor motoneurons and inhibit the extensor inhibitor.

These experiments demonstrate the ability of single central interneurons to release reciprocal motor output involving many efferent units, and they strengthen the conclusions of others (*4*) that complex, cyclical effects may be initiated by single cells. We have used similar techniques of stimulation in an attempt to reveal other properties of these command fibers and, in particular, to explain why a number of elements (probably a dozen or more) yield the same category of effect. Two findings are of particular interest. First, the influence of some elements extends beyond a single motor system. For example, several also produce swimmeret movements and may be identical with the ones described in that context by Wiersma and Ikeda (*4*). In these cases the effect upon the appendages may be ipsilateral, whereas the output to the abdominal muscles is always bilaterally symmetrical. Second, almost all of the command fibers controlling abdominal posture affect several segments, differing from one another in the segmental ratio of their output strength. Some, for example, produce strong discharges caudally and weak ones rostrally, while others have the reverse distribution. We have analyzed this property

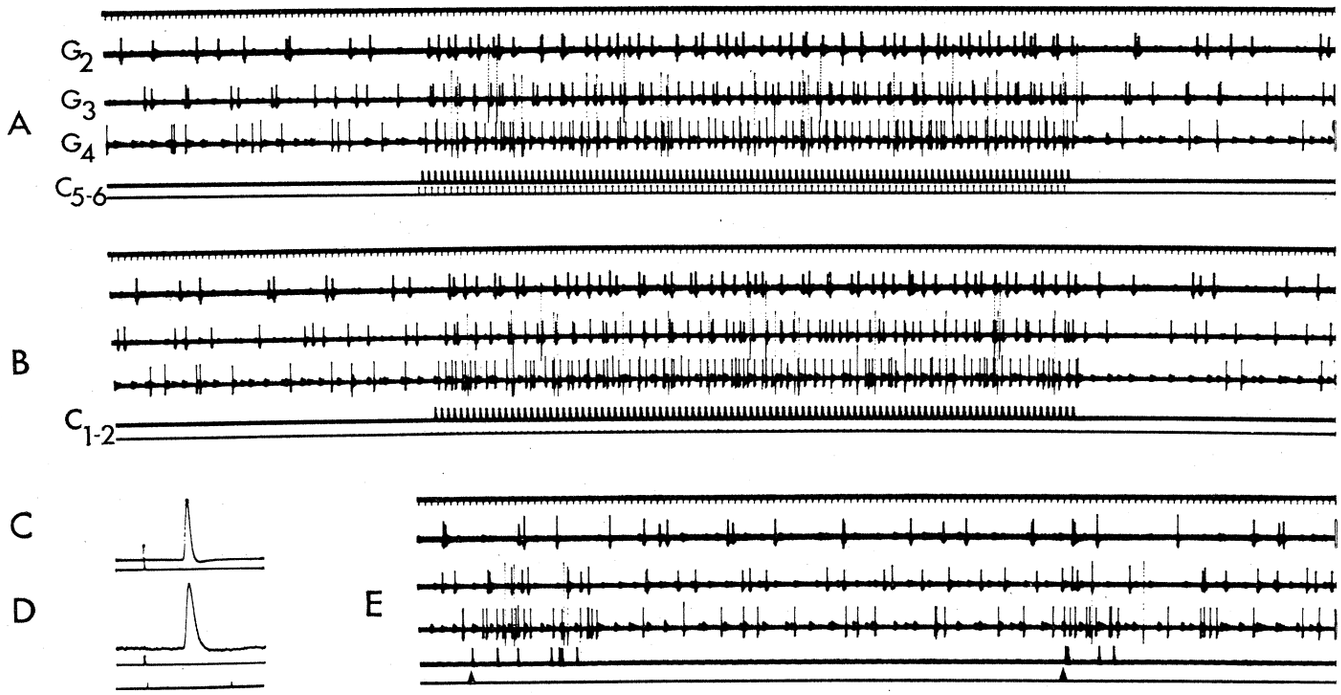

Fig. 2. (A) Effect of stimulating a flexor command fiber at the rostral site. The top trace is a 10-msec time mark; the next three (G_{2-4}) are records from the slow-flexor motor roots of ganglia 2, 3, and 4; the fifth is a record from the other end of the interneuron, at the caudal site; and the bottom trace is a stimulus monitor. (B) Fifth trace now records from the rostral filament that was stimulated in A; the caudal end is stimulated here. As in A, a train of all-or-none impulses in the recorded filament accompanies the motor effect. (C and D) Records of the impulse recorded in the caudal and rostral filaments, respectively, upon stimulation of the other end. Time mark, 10 msec. (E) Response of the interneuron at the rostral site to sensory stimulation before caudal isolation. At arrows, uropod blades were passively flexed. Recording as in A and B, with signals from the rostral site at higher amplification.

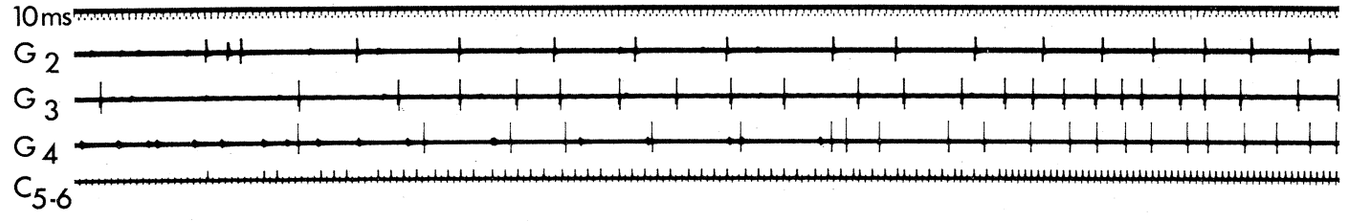

Fig. 3. Effect of stimulating an extensor command fiber at gradually increasing intensity. Traces as in Fig. 2; continuous stimulation of the rostral filament at 100 per second with gradually increasing intensity. The unit that responds in the root traces is the flexor inhibitor; all motoneurons are inhibited.

by filming the movements of the unrestrained abdomen in response to command-fiber stimulation. The results confirm that individual elements produce movements that differ from one another in the degree to which particular segments are involved (*9*). Each command fiber in a particular category thus may code for a specific and unique abdominal geometry.

In summary, experiments in which the same central neuron is isolated at two different points and stimulated at either of these have shown that repetitive activity in a single cell can release reciprocally organized, tonic motor output involving well over 100 efferent elements. The sensory inputs to such command fibers can be correlated with the motor effects produced by the latter. Central neurons producing similar abdominal movements differ in their influences upon other motor systems and also in the segmental distribution of their effects. It is likely that in producing such sterotyped output patterns, command fibers merely trigger activity in a series of ganglionic centers whose own organization ensures reciprocity and symmetry.

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