Crayfish Escape Behavior and Central Synapses. I. Neural Circuit Exciting Lateral Giant Fiber

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CRAYFISH ARE SOUGHT as food by fish, birds, amphibia, and mammals, as well as man (54, p. 460). They commonly escape these predators by darting backward and away from an enemy on contact, or as the predator approaches. This maneuver is accomplished by a sudden flexion of the tail, exerting a thrust backward and sometimes upward against the aquatic medium. A single tail flexion, commonly called a tail flip or flick, often suffices; a more prolonged mode of escape, swimming, consists of a periodic sequence of abdominal flexions and extensions.

In 1947, Wiersma (67) showed that stimulation of any single lateral or medial giant fiber of the nerve cord led to a rapid abdominal flexion. Recently it has been shown that the giant fibers excite all of the fast flexor motoneurons of the abdomen which innervate the phasic flexor muscles (37, 38, 61). It has been assumed from this evidence that the giant fibers are the sole command or decision fibers responsible for eliciting escape behavior. Schrameck (60) has shown, however, that many tail flicks—including most of those in swimming—are accompanied by giant fiber spikes in intact, unrestrained, chronically implanted animals. Nevertheless, recent experiments by Wine and Krasne (71) show that a phasic mechanical stimulus to the tail leads to tail flicks that are always mediated by lateral giant fiber activity. For this particular input, therefore, the lateral giant is a critical decision fiber for the behavior. Krasne has also found that repetition of the stimulus as slowly as once per minute reveals a lability in the response, which fails to follow an increasing proportion of the stimuli. The behavior thus habituates to infrequently repeated stimuli. However, since prolonged stimulation of the lateral giant at frequencies up to 1 Hz results in the continued appearance of tail flicks (38, 41), the response lability must be located in the pathways afferent to the giant fiber, or in that neuron itself.

In 1960, Kao (25) recorded slow depolarizing potentials in the lateral giant axon to shocks delivered to afferent roots. The potentials could elicit spikes, and were therefore excitatory postsynaptic potentials (EPSPs). Krasne (40) showed that on repetition of a stimulus these EPSPs waned with a time course similar to behavioral habituation. This discovery represented an important neural correlate for an adaptive behavior, and it seemed that to the extent that the properties of the EPSP could be explained, the behavioral lability could be explained in physiological terms.

This paper describes the results of an investigation of the pathways responsible for the activation of the lateral giant. The second paper (73) locates and analyzes the points in the circuit responsible for the behavioral habituation. The final paper (74) describes the mechanism of activation of the fast flexor motoneurons by the lateral giant fiber.

METHODS

Procambarus clarkii were used for all experiments. Male and female specimens about 8 cm long were obtained from H. A. Dahl Co., Berkeley, Calif., and maintained in aerated tapwater tanks at room temperature until use.

Before dissection the animals were immobilized by cooling briefly in ice. A strip of dorsal carapace and hypodermis about 5 mm wide was removed from the abdominal segments 1

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through 6, and the underlying intestine was excised. The fast flexor muscles were separated along the midline, and the thorax and head were removed and discarded. The abdomen was pinned ventral side up in a Lucite dish containing two ledges of wax, and filled with cold oxygenated van Harreveld's solution (66), with the bicarbonate buffer replaced with Trisma at pH = 7.2. Mirrors placed in the groove between the wax ledges and under the abdomen reflected a focused light beam either directly or obliquely through the abdomen. The ventral nerve cord was exposed by removing the overlying ribs, cuticle, and hypodermis. The remaining cross connections between the flexor muscles, particularly the transverse muscles (55), were cut and trimmed, and the two sides of the abdomen were separated slightly so that the nerve cord was well illuminated by the light reflected from below. The ventral blood vessel was removed from the nerve cord, and the connective tissue sheath was peeled off the 2/3, 4/5, and 5/6 connectives, from which single axons were dissected with fine needles and forceps for stimulation and recording. Oblique transmitted light, directed nearly along the nerve cord axis, was found best for visualizing and separating single axons. For intracellular studies, the third or fourth ganglion was desheathed and perineural tissue washed away with a jet of saline (51). Cell bodies and other ganglionic landmarks are seen most easily in direct transmitted light. In experiments where the giant fibers might be stimulated, all third roots and sixth ganglion roots containing phasic motoneurons (45) were cut to prevent twitching of the flexor muscles. The temperature of the bath was maintained at 18 C by a Peltier cooling unit placed under the preparation chamber.

Stimulation and recording from single axons, roots, or the nerve cord was accomplished by the use of micromanipulated suction electrodes (52), connected to a switch box which allowed either of two stimulators to be connected to any electrode, or any electrode could be switched to one of four Tektronix a-c preamplifiers. All signals were displayed on a conventional oscilloscope and photographed. Intracellular recordings were made with micropipettes pulled to fine tips less than 0.5 μ in diameter and filled with 3 M KCl. The resistance was between 10 and 40 megohms. The desired elements were most easily penetrated by approaching the ganglion obliquely in the vertical plane including the nerve cord.

Signals were recorded using a Bak capacitance-compensated high-impedance d-c preamplifier (Electronics for Life Sciences, Rockville, Md.), used differentially. This unit is provided with a Wheatstone bridge circuit for passing current through the recording electrode, and this provision was frequently employed to stimulate cells or control their level of polarization (15). Stimulus currents were isolated from ground with a W-P Instruments (Hamden, Conn.) photoelectric isolator. The only ground introduced into the recording system was by a lead to the bath. Extracellular stimuli were obtained from Tektronix pulse-generating equipment, and isolated with transformers.

Procedures for locating and impaling desired elements, minimizing the utilization time of stimulation, and calculating the arrival time of spikes in presynaptic elements are described in RESULTS.

RESULTS

Lateral giant response to afferent volleys: component analysis

When an electrical stimulus is applied to the first or second root of an abdominal ganglion, a depolarizing potential may be recorded from the ipsilateral lateral giant neuron in the segment stimulated. This response is best seen in penetrations of the giant axon just anterior to a ganglion, rostral to the septal synapse with the next posterior lateral giant axon and the point where the major dendrite arches medially and ventrally into the ganglionic neuropil. Such a recording is illustrated in Fig. 1. A shock was delivered to the second root of the fourth ganglion; the ipsilateral lateral giant

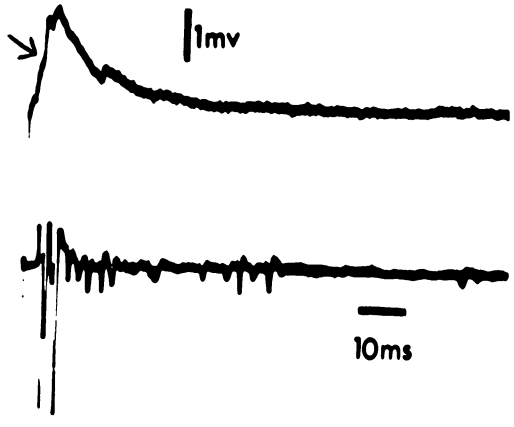

FIG. 1. Response recorded intracellularly (upper trace) from the lateral giant axon in the fourth ganglion to stimulation of the second root of the same segment. The lower trace is an extracellular recording from the ipsilateral ventral nerve cord, 4/5 connective. The arrow marks the peak of the early component of the response.

axon was penetrated just anterior to the fourth ganglion, and the ventral nerve cord monitored on the ipsilateral 4/5 connective. This lateral giant depolarization is the compound excitatory postsynaptic potential (EPSP) reported by Kao (25) and Krasne (40). The inflection on the rising phase corresponds to the peak of the first component of the response, designated the α-component (40). The peak and shoulder of the response correspond to Krasne's late β- and γ-components.

The maximal compound EPSPs recorded from the lateral giant axon in over 10 experiments were quite small, always under 5 mv and frequently less than 2 mv. In order to analyze unitary components of the response, penetrations of the lateral giant dendrite were made close to the probable synaptic sites, using the reconstructions made from dye injections by Remler et al. (58) and Selverston and Kennedy (61). The procedure used (see Fig. 2) involved the following steps: The lateral giant axon was isolated for stimulation by teasing it free from the 5/6 or 1/2 connective, and its response was monitored by an electrode placed on a connective next to the ganglion being studied. While repetitively stimulating the giant fiber at 2 Hz, the microelectrode was lowered into ganglionic neuropil near the ventral ipsilateral branch of the lateral giant dendrite at its lateral bifurcation, until a region was found bearing a small triphasic focal potential corresponding to the antidromic invasion of the dendrite by the evoked impulse. Probing in this region, the electrode was positioned so as to maximize this potential; whereupon a light tap occasionally resulted in a successful penetration of the dendrite of the giant fiber (cf. ref 8). Two electrodes were placed on the relevant afferent root (usually the second), one for stimulation and one for recording. To observe the effects of controlled activity in identified interneurons on the lateral giant, the interneuron was teased free from the nerve cord at least one segment away from the ganglion of penetration for stimulation, and its activity was monitored by the electrode on the nerve cord.

This procedure yielded recordings of subthreshold potentials in giant fibers in over 40 specimens. The EPSPs were of much greater magnitude than those recorded from the axon or proximal dendrite. Figure 3 illustrates several features of typical responses to repetitive electrical stimulation of the second root. First, the α-component reliably maintains its initial amplitude at 10 Hz. The late components are quite labile at this frequency, and decline rapidly to less than half their original amplitude (40). Finally, since the compound EPSPs failed to evolve a spike, the threshold of the lateral giant cell is extraordinarily high as seen from the dendrite, exceeding 50 mv in this case. This contrasts with the 7- to 8-mv threshold measured from axonal recordings

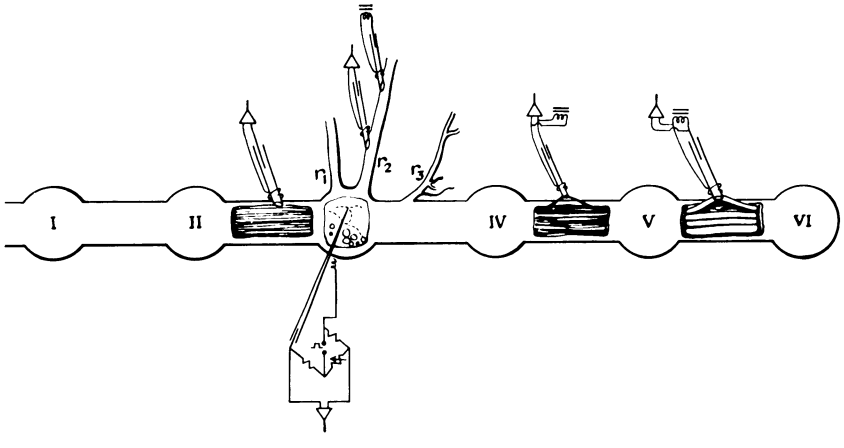

FIG. 2. Typical layout of electrodes and nerve cord used for these experiments. The nerve cord is left connected by its roots to the abdomen (not shown). Roman numerals refer to abdominal segments; r_1 is the first root, etc. See METHODS and the text for description of dissection, equipment, and procedures.

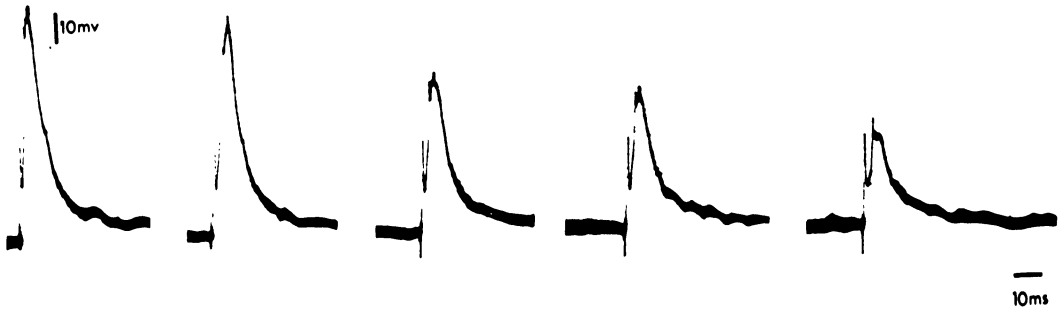

FIG. 3. Lateral giant dendrite responses in the fifth ganglion to repetitive stimulation of the second root of the same segment at 10 Hz. The 1st, 2nd, 5th, 10th, and 30th responses are shown from left to right. The initial downward deflection is the stimulus artifact. The first fast depolarization is the α-component of the response; the slower, later wave is the β-component. A separate γ-component is not present in these responses.

(40). Part of this difference may be due to damage inflected by the dissection, but most of it is attributable to the difference expected in recording from one or the other of the two spatially distinct regions: the synaptic zone and the spike-initiating site (see DISCUSSION).

Increasing the intensity of single shocks delivered to afferent roots results in graded augmentation of both early and late components (Fig. 4). This effect of recruiting additional sensory axons indicates that there are a variety of presynaptic elements that excite the lateral giant and contribute to both phases of the response. Very weak stimuli can elicit either an α- or β-component in isolation, testifying to their independence. Before exploring the detailed composition of these components, it is necessary to answer the more general question: which afferent systems contribute to the pathways exciting the lateral giant neuron?

Sensory modalities exciting lateral giant

To identify the sensory pathways activating the giant cell, an electrode was placed in the dendrite and receptors from each sensory modality were stimulated in turn by their adequate stimuli while monitoring the sensory response in the appropriate afferent roots. The carapace tactile receptors (47) were found to provide very strong excitation to the lateral giant in over 20 experiments. The largest responses were elicited by natural stimuli to the ipsilateral first or second root fields of the ganglion (69) in which the giant was penetrated; however, responses in one ganglion could be seen to stimulation of any abdominal segment. Depolarization in lateral giant fibers could be elicited by brushing the hairs or by air bubbles blown from a pipette and allowed to strike the pleural plates of the same and next posterior segment to that recorded (the second root field). The responses to such stimuli (Fig. 5*A*) resembled in their shape and amplitude the potentials evoked by second root shocks. Occasionally, in freshly dissected preparations, such stimuli elicited escape responses. Finally, in several animals it was possible to stimulate single hairs naturally and observe their unitary EPSPs in the lateral giant. One such experiment is shown in Fig. 5*B*.

Stimulation of the cutaneous pressure receptors of the ventral soft cuticle (52), the sensory setae of the swimmerets (7), or the proprioceptors in the appendages (1, 7, 69) sometimes evoked a few depolarizing deflections in the lateral giant. Stimulation of these receptors invariably involved local water currents or slight vibrations, however, and it is likely that these excited some tactile hairs, which in turn caused the responses observed. The ventral nerve cord stretch receptors (24, 34) can be activated by extending the telson; this operation had no effect on the giant fiber. In five preparations, the pleural plates were trimmed off, the ribs cracked at their lateral junctions with the carapace, and the half dorsal carapace of all segments rotated around a longitudinal axis through the swimmeret coxa so

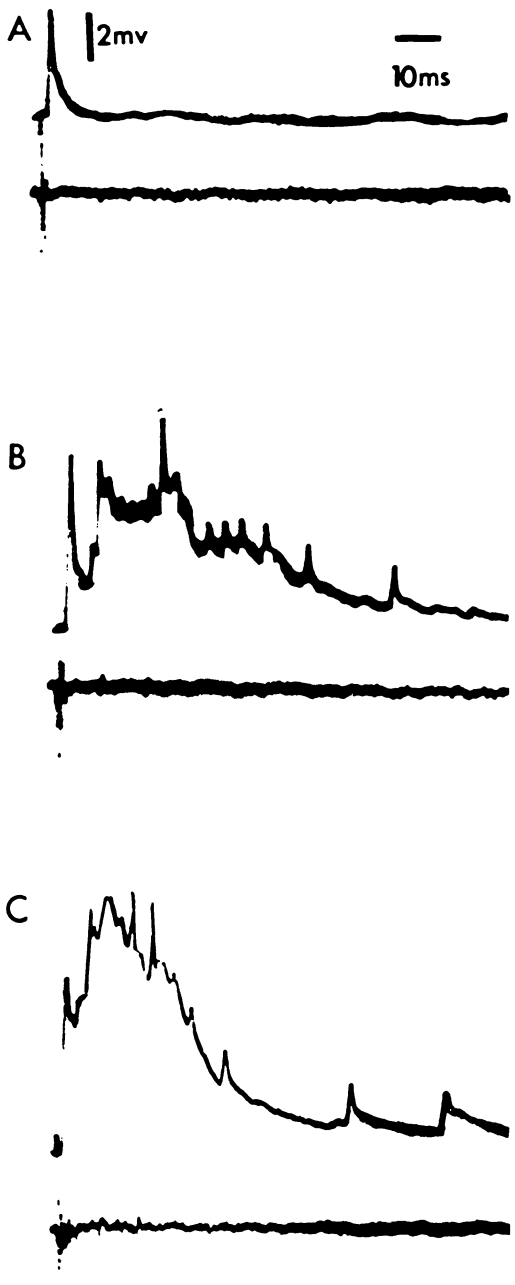

FIG. 4. Lateral giant dendrite responses to increasing numbers (*A* to *C*) of afferent fibers recruited by increasing the intensity of a shock delivered to the second root. Stimulation and recording from the fourth segment. Upper trace is intracellular record; lower trace is a monitor of the afferent volley recorded by a proximal root electrode.

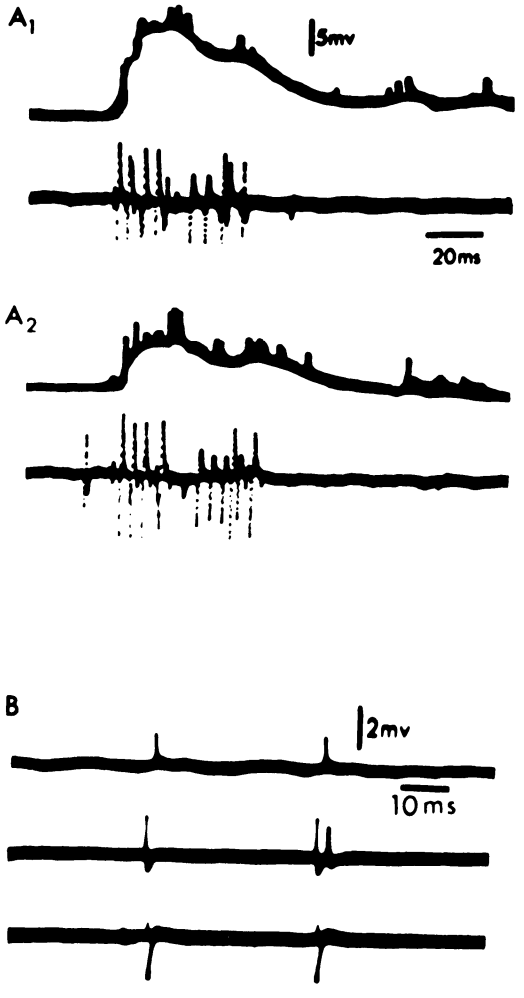

FIG. 5. Lateral giant dendrite responses to tactile stimulation of hairs on the ipsilateral pleural plate of the same and next posterior segments. *A:* stimuli consisted of air bubbles striking the carapace. Upper trace is intracellular recording from the fourth ganglion; lower trace is monitor of afferent activity in the second root to the same ganglion. A_1 and A_2 are the first and third of a series of stimuli delivered at 0.5 Hz. The late part of the EPSP declines on repetition of the stimulus. *B:* from a different animal. Unitary EPSP in the lateral giant dendrite (upper trace) caused by discharges in a single tactile receptor on the ipsilateral fourth pleural plate. The afferent spike is recorded in the second root (middle trace) and in the nerve cord, 2/3 connective. The receptor was excited by gentle water currents.

that both the ventral surface of the nerve cord and the dorsum of the animal were facing upward. The muscle receptor organs (11, 13) of the same and next posterior segments were exposed (see ref 10 for dissection) and stretched with hooks; no response was observed in the lateral giant, even when other reflexes controlled by these re-

ceptors were obtained (14). In the same experiments, the dorsal nerve branch containing the stretch receptor afferents was stimulated electrically; or else the branch to the phasic extensor muscles was stimulated. In both cases a second root monitor provided evidence that all axons present in the stimulated branch were responding; in neither case was a response seen in the lateral giant. This experiment eliminates the possibility of recurrent activation of the giant fiber on stimulation of extensor motoneurons. Finally, the caudal photoreceptor apparently does not excite the lateral giant, since it would be firing regularly under the conditions of these experiments (31) and would have led to the appearance of periodic EPSPs in the giant, which were never observed. It may be concluded, then, that the only significant sensory pathway activating the lateral giant escape system is the tactile hair population, at least in the third and fourth abdominal segments. Similar conclusions arise from the recent behavioral experiments of Wine and Krasne (71) and Larimer et al. (46), who also show that there is no input to this system from the head or most of the thorax. Thus, stimulation of an afferent root is approximately functionally equivalent to delivering a natural phasic tactile stimulus to the crayfish carapace or the medium surrounding it.

Properties of excitatory synapses onto lateral giant

EARLY COMPONENTS. The first part of the lateral giant response to afferent stimulation consists of monosynaptic electrically induced PSPs from tactile afferent fibers. Figure 6 illustrates the effects of these direct connections. In *A,* the first hump in the lateral giant response to weak electrical

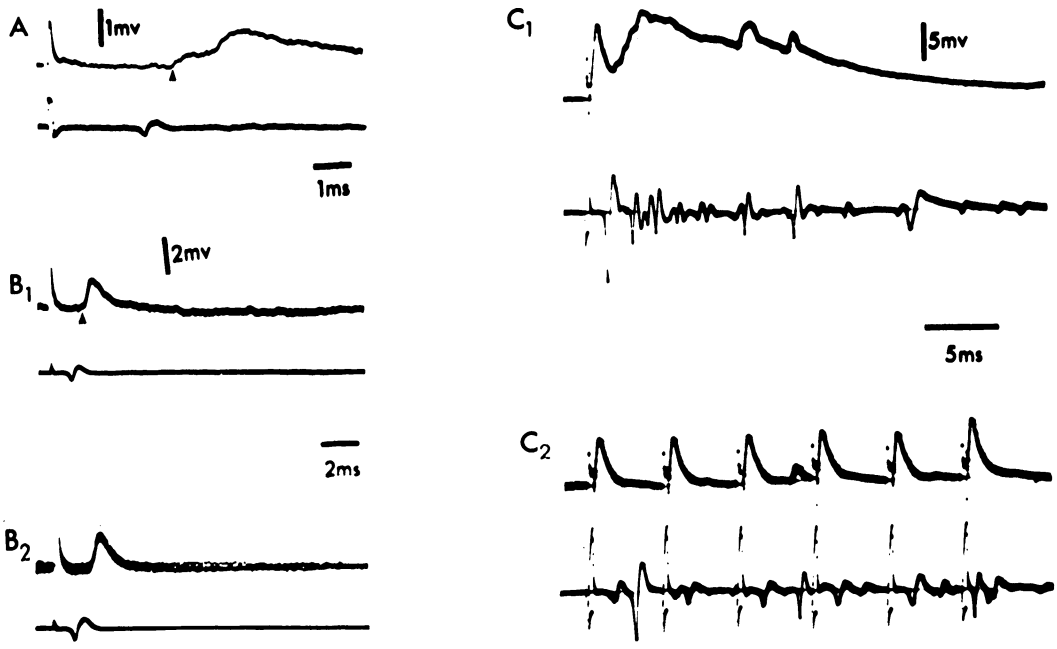

FIG. 6. Properties of the early component of the lateral giant response. Upper trace is from the lateral giant dendrite in the third (*A*) or fourth (*B* and *C*) ganglion; lower trace is a monitor on the ipsilateral second root to the same ganglion (*A* and *B*) or a record of nerve cord activity in the 2/3 connective (*C*). *A:* stimulation of the second root excites two afferents (only one is recorded by the root monitor) which elicit two EPSPs in the lateral giant. *B:* in another animal, the first afferent stimulated in the second root elicits a unitary electrical EPSP in the lateral giant. B_1 is a single response; B_2 shows superimposed responses to stimuli delivered at 10 Hz. Triangles on the upper traces point to the calculated arrival time of the presynaptic afferent spike (see text). In *C,* from a different preparation, the late response to maximal second root stimulation (C_1) is blocked at a repetition rate of 200 Hz, leaving the early component undiminished (C_2).

second root stimulation always corresponded to the presence of the first afferent impulse recorded in the root monitor. (The second hump of the response is due to a fiber in the root whose spike height was too small to be recorded through the root monitor at the gain used.) The triangle points to the calculated arrival time of the afferent spike seen below. This instant was calculated by measuring the distances between the two root electrodes and the microelectrode (see Fig. 2), measuring the conduction velocity between the stimulating and recording root electrodes and, assuming this to be constant (see below), extrapolating the arrival time in the ganglion.

This technique is subject to two sources of error. *1*) The interval between stimulation and passage of the afferent spike past the root monitor includes an unmeasured utilization time for stimulation. Normally, this utilization time is minimized by using very brief, intense stimuli. However, this procedure cannot be used here because such a stimulus would recruit many other afferent spikes in the root. Instead, a just suprathreshold, long stimulus was employed, and the latency was measured from the end of the stimulus pulse. If different pulses were chosen for stimulation, with longer and weaker shocks, the interval increased by increments corresponding to the increments in stimulus duration. Furthermore, if a long shock adjusted as described above were greatly increased in strength, the latency was shortened by the duration of the stimulus. Thus with the procedure employed, the utilization time corresponds to the stimulus duration, and it is eliminated from the calculations by measuring latencies from the end of the shock.

2) The assumption of constant afferent velocity might be incorrect. In particular, it seems possible that the central afferent processes are thinner than the peripheral axon. This would cause the afferent impulse to arrive later than shown in the figures. In consequence, this error would cause measurements of synaptic delay, the latency between the calculated arrival time of the presynaptic impulse and the foot of the postsynaptic potential, to be overestimated. However, there is evidence that this error is negligible. On two occasions, a tactile afferent terminal was penetrated in a ganglion near to an interneuron onto which it synapsed. Stimulating the sensory axon through a distal root electrode, while recording an impulse from a proximal root electrode as well as the central process, the central and peripheral conduction velocities were calculated and found to be identical in both experiments.

The brief postsynaptic potential associated with the afferent impulse in Fig. 6*A* begins at the moment the presynaptic spike arrives at the region of the microelectrode penetration. Thus, the synaptic delay is essentially zero. Figure 6B_1 shows another afferent EPSP, with no apparent synaptic delay; it consists of a rapid potential following each spike in an afferent fiber, excited by stimulating the second root. In Fig. 6B_2, the responses to successive stimuli at 10 Hz are photographically superimposed to show that the response is uniform in latency, amplitude, and duration. These responses represent unitary elements of the α-component to a maximal root shock shown in Fig. 6*C*. When shocks are delivered at 200 Hz, the late components drop out entirely, leaving the α-component unchanged. These properties of the members of the α-component were observed in over 20 preparations: one-to-one correspondence with single afferent impulses, all-or-none and constant shape, brief duration, very short latency, and high-frequency following, lead together to the conclusion that the α-component is the sum of unitary, monosynaptic, electrically induced EPSPs between tactile afferents and the lateral giant.

It is known (69) that at least some of the tactile afferents entering a ganglion send branches that ascend or descend in the nerve cord to the next ganglion. It is therefore frequently possible to record an afferent volley in the root before it enters the ganglion and in the cord just caudal to the ganglion, and to obtain a more accurate estimate of arrival time of the volley in the ganglion. Such an experiment is illustrated in Fig. 7, and shows that the α-component of the response to a maximal root shock begins at the moment of arrival of the presynaptic spikes in the ganglion.

LATE COMPONENTS. The late components of the lateral giant response to afferent activity are very different in nature from the early component. Krasne (40) showed that the β- and γ-components are very variable in shape, wane on repetition of the stimulus, are of longer latency than the α-component, and seem to consist largely of shifting

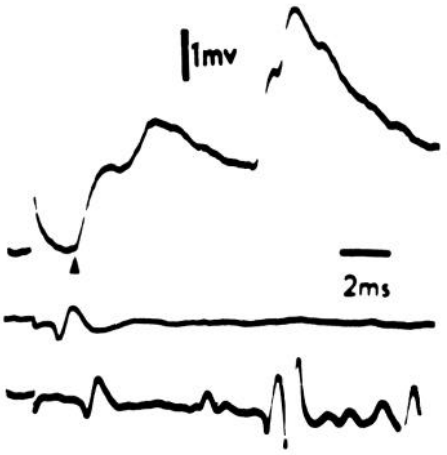

FIG. 7. Absence of synaptic delay of early component of lateral giant EPSP, shown by interpolating the arrival time of the afferent volley in the ganglion (triangle in the figure) between its arrival times in the root monitor (middle trace) and the nerve cord in the 4/5 connective (bottom trace). Upper trace is from the lateral giant dendrite in the fourth ganglion, whose second root was stimulated.

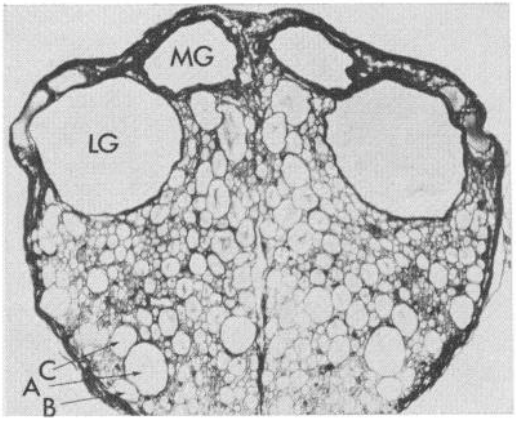

FIG. 8. Cross section of the ventral nerve cord in the 3/4 connective; 10-μ section, embedded in paraffin, stained with Masson's trichrome. Dorsal surface is up. MG: medial giant; LG: lateral giant; A, B, and C: identified tactile interneurons (see text). (Photograph courtesy of D. Kennedy.)

unitary transient components which drop out with repetitive stimulation as the β- and γ-components decline (see Figs. 1, 3–5, 6*C*, and 7). Since these properties suggested that the late components might be polysynaptic, it was decided to investigate the possibility that interneurons excite the lateral giant.

Since the only pathways exciting the lateral giant cell are tactile ones, interneurons exciting the giant, if they exist, must be abdominal tactile interneurons. A variety of such interneurons have been described in crayfish (68, 69). Single neurons can be identified and characterized by their receptive fields, locations, sizes, and response properties, and the same fibers can be located again and again in different animals, always having the same distinct and unique set of properties. Three such neurons which were studied extensively in these experiments are indicated in the cross section of the ventral nerve cord shown in Fig. 8. Interneuron A (A6 in ref 69) is a unisegmental tactile interneuron which has recently been studied extensively by Kennedy (33). Its receptive field is the dorsal surface of the ipsilateral telson and uropods. Interneuron B (A63) is a multisegmental tactile interneuron with a receptive field of tactile hairs on the ipsilateral second through fourth segments. Interneuron C (A64) is a multisegmental tactile interneuron responsive to tactile stimulation of the entire abdomen.

In thirteen experiments, one of the above interneurons was dissected free from the nerve cord with fine needles and its influence on the lateral giant was tested by stimulating it while recording from the giant neuron. Interneurons A, B, and C, as well as others, did produce all-or-none, brief depolarizing potentials in the giant. Such a potential is seen as the second deflection in the first trace of Fig. 9.

Before proceeding with a detailed analysis of these connections, it seemed important to establish that such potentials were involved in the late response of the giant cell to peripheral stimulation. In Fig. 9, shocks were delivered to the second root and to interneuron C, and the interval between the shocks was varied to observe the interaction between the two lateral giant responses. When a peak in the β-component of the root response preceded the stimulus to the interneuron by 3 msec or less, the response to the interneuron spike was blocked (third and fourth traces). Furthermore, when the interneuron spike preceded the usual time of the peak of the β-component by 3 msec or less, the peak failed to appear. This result, obtained in several animals, in-

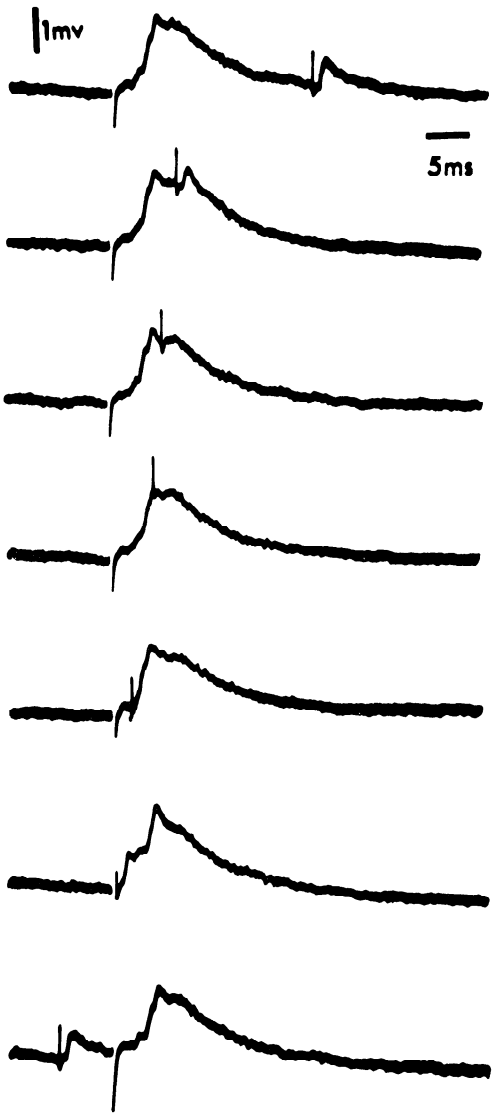

FIG. 9. Interaction between lateral giant responses to second root volleys and spikes in tactile interneuron C. The response to second root stimulation is preceded by a downward-going artifact; the interneuron response is preceded by an upward-going artifact. Recordings are from the lateral giant dendrite in the fourth ganglion. Stimuli were applied to the ipsilateral second root to that ganglion, and to the interneuron axon in the 4/5 connective.

dicates that occlusion has occurred and that interneuron C is therefore part of a pathway shared by both direct stimuli and root stimuli, where the lateral giant is the final common element of the pathway (62).

Figure 10 presents more direct evidence that tactile interneurons constitute an important pathway of activation of the lateral giant in response to peripheral stimulation. In Fig. 10*A*, interneuron A has been isolated on the middle trace, and its activity monitored there and in the cord monitor. Natural stimulation in its receptive field (brushing the telson) elicits spikes in the interneuron which are always followed by large depolarizing potentials in the lateral giant. These synaptic potentials can be seen in the third, fourth, and fifth, but not the sixth ganglion. So connections between interneuron A and the giant fiber are repeated in most of the abdominal segments. In Fig. $10B_1$ the same experiment has been repeated with interneuron C. Connections between interneuron C and the lateral giant also occur in several ganglia. In Fig. $10B_2$, it is evident that a large part of the late response of the giant cell to second root stimulation consists of EPSPs generated by repetitive activity in interneuron C. In another preparation (Fig. 10*C*), it is shown that interneuron C contributes to both the β- and γ-components of the lateral giant response. Indeed, the very existence of separable β- and γ-components depends on the double-burst nature of firing in many tactile interneurons. Frequently, a phasic stimulus elicits spikes in these interneurons which cluster into two bursts and then the late component of the lateral giant response has two separate phases. When such clustering of interneuron firing does not occur, a separate γ-component is not discernible.

These results have been obtained in 15 animals for cells A, B, and C, as well as other identified tactile interneurons. They indicate that there exists a fairly large population of tactile interneurons with different and often overlapping receptive fields which excite the lateral giant in each abdominal segment 2 through 5.

Since these identified interneurons contribute so strongly to the normal activation of the lateral giant by natural stimulation, it is relevant to explore the properties of their synapses. Figure 11 illustrates the results of a study of the synapse from interneuron A. Electrical stimulation of the isolated interneuron led to a large rapid depolarization (8 mv) in the giant cell. After withdrawing the microelectrode and increasing the gain 10-fold, a focal potential

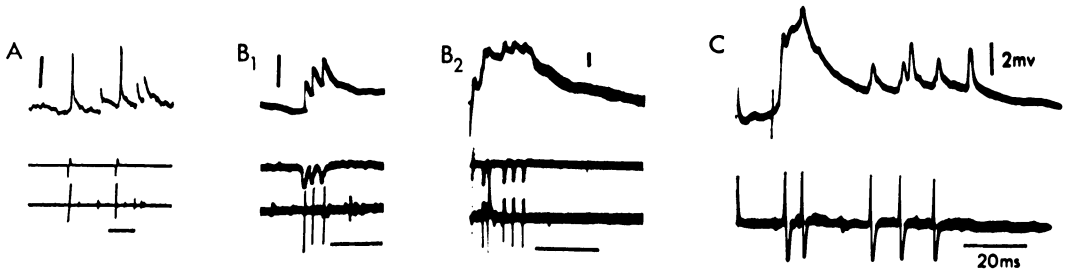

FIG. 10. Identification of tactile interneurons exciting the lateral giant. Records are from four different animals. Upper trace is microelectrode recording from the lateral giant dendrite in the fourth (*A* and *B*) or third (*C*) ganglion. Middle trace (in *A* and *B*) records activity in an isolated interneuron axon from the 5/6 (*A* and B_1) or 4/5 (B_2) connective. Bottom trace is a nerve cord monitor on the 3/4 (*A* and *B*) or 2/3 (*C*) connective. *A:* spikes in tactile interneuron A, elicited by natural stimulation in its receptive field, generate EPSPs in the giant neuron. B_1 shows the same result when interneuron C is activated tactilely. In B_2 and *C*, second root shocks in the segment of penetration elicit activity in interneuron C which contributes to the β- and γ-components of the lateral giant response. (Extracellular spikes retouched.)

representing the passage of the presynaptic spike in the interneuron was recorded on stimulating the interneuron. This gives a very precise measure of the arrival time of the presynaptic spike in the ganglion (cf. ref 27), and it may be seen that there is no measurable synaptic delay to the foot of the EPSP. This synaptic transmission is also stable for many minutes at stimulation rates up to 400 Hz, a property seen in no chemically transmitting synapse.

Figure 12 illustrates properties of the synapse between interneuron C and the lateral giant. In Fig. 12*A*, the all-or-none, brief EPSP following stimulation of the interneuron appears in the giant dendrite with no delay following the calculated arrival time of the presynaptic impulse. It also follows stimulation at 400 Hz; many superimposed responses in Fig. 12*B* show that the shape is constant and that there is no latency jitter. By the same criteria applied to the members of the α-component, it is concluded from over 10 similar experiments that these tactile interneurons electrically excite the lateral giant in each of several abdominal ganglia.

Figure 13 presents another result consistent with the notion that the early and late responses of the lateral giant consist of electrical EPSPs. It was often observed that depolarizing the lateral giant membrane by passing current outward through the recording microelectrode had little effect on the response, while strong hyperpolarization reduced the peaks of the response by blocking some of the unitary components. This result is the opposite from that found in chemically mediated synapses (9), but may occur in electrical synapses if the hyperpolarization spreads to the presynaptic elements and blocks spikes in some of them (2). Finally, it is significant that it has been impossible to demonstrate any change in the input resistance of the lateral giant dendrite during any portion of the response to peripheral stimulation (see ref 73).

To summarize, convergent monosynaptic and polysynaptic electrical excitation onto the lateral giant summates to form a large compound response to peripheral stimulation. When this potential exceeds threshold (Fig. 14), a spike is evoked and the animal produces an escape response. The escape behavior is as all-or-none as the impulse in the giant fiber (46, 67, 71), which thus constitutes a "decision fiber" for this behavioral response to phasic abdominal tactile stimulation. The orthodromic spike riding on the compound EPSP in the lateral giant (Fig. 14*A*) attains about the same peak voltage as an antidromic impulse recorded in the lateral giant dendrite (Fig. 14*B*).

Activation of tactile interneurons

DIRECT AFFERENT EXCITATION. Since most of the excitation of the lateral giant is polysynaptic and mediated by tactile interneurons, the activation of these cells was studied in 25 experiments in order to com-

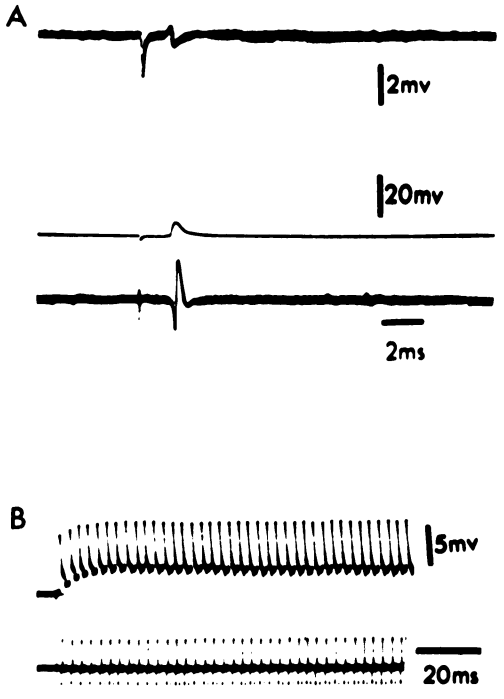

FIG. 11. Properties of the EPSP in the lateral giant from interneuron A. *A:* response recorded from the giant cell dendrite in the fourth ganglion (middle trace) to stimulation of the interneuron axon in the 5/6 connective. The microelectrode was withdrawn and the gain increased in the top trace, which records the field potential generated by a spike in interneuron A passing the site of the microelectrode. This gives a measure of the presynaptic spike arrival time, and also the membrane potential of the lateral giant dendrite (97 mv). *B:* in the same preparation, the EPSP follows with no decrement long trains of presynaptic stimulation at 400 Hz. In *A* and *B,* the interneuron activity is monitored on the bottom trace from the 3/4 connective.

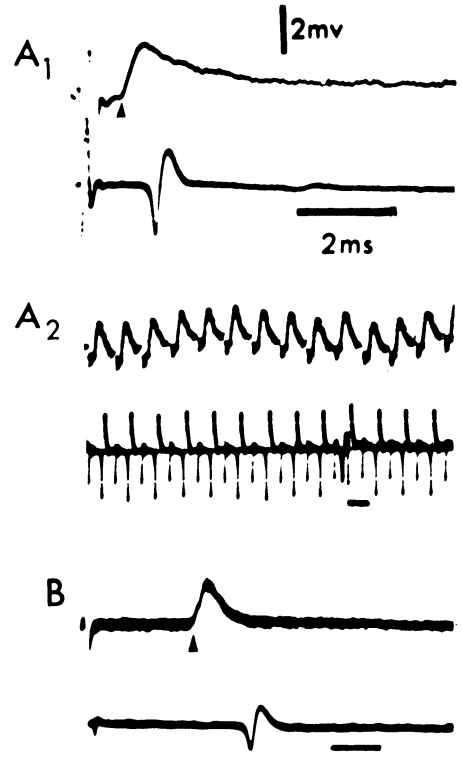

FIG. 12. Properties of the EPSP in the lateral giant from interneuron C. Responses recorded from the giant dendrite (upper trace) in the fourth (*A*) or third (*B*) ganglion to stimulation of the interneuron axon in the 4/5 connective. The interneuron activity in the 3/4 (*A*) or 2/3 (*B*) connective is shown on the bottom trace. The response to high frequency (400 Hz) stimulation (A_2) is the same as the response to single presynaptic spikes (A_1). *B:* superimposed responses to stimuli repeated at 10 Hz. The triangle marks the calculated arrival time of the presynaptic spike at the junction.

plete the circuit of the lateral giant-mediated escape response. The integrative properties, activity patterns, input organization, and discharge mechanisms of tactile interneurons as a class have been studied extensively (35, 36, 56, 63), but these properties have not been examined in individually identified units, except for the recent experiments on interneuron A (33). In the following experiments, tactile interneurons were studied as single units, to determine which of the many properties of the class of all interneurons they share.

Tactile interneurons were studied in much the same manner as the lateral giant neuron.

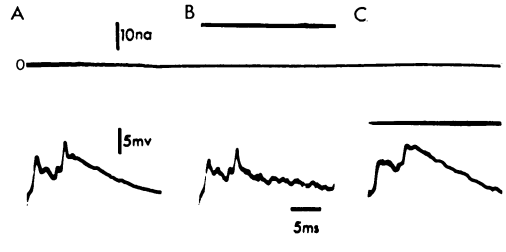

FIG. 13. Effects of polarizing currents on the lateral giant dendrite response. The upper trace records the current which was passed continuously through the recording electrode; 0 marks the current base line. The bottom trace is the intracellular recording from the fourth ganglion, whose second root was stimulated. Depolarization (*B*) has little effect on the control response (*A*); hyperpolarization (*C*) blocks some of the unitary electrical EPSPs.

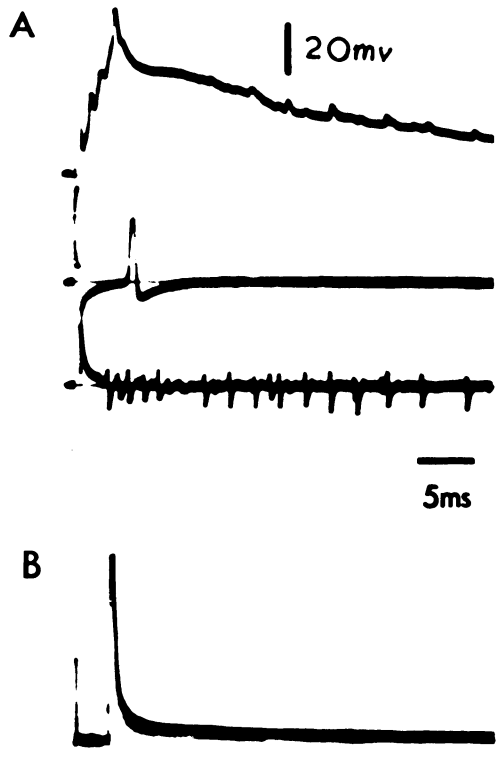

FIG. 14. Suprathreshold orthodromic activation of the lateral giant. In *A*, the upper trace records the intracellular potential in the dendrite in the third ganglion, whose second root was stimulated. The lateral giant axon was monitored in the 2/3 connective on the middle trace, and the cord activity was recorded from the 5/6 connective in the bottom trace. *B:* antidromic spike recorded from the same dendritic locus as in *A*.

Some of them have been injected with fluorescent dye (33, 75) and the general location of their dendritic trees is known. Usually, an interneuron was isolated from the 4/5 connective of the nerve cord, identified by its size, position, receptive field, and response properties, and penetrated in the third and fourth ganglion, using its focal potential during antidromic impulse generation as a guide. The interneuron was also monitored in the 2/3 connective. Judging from the large spike height and large synaptic potentials, penetrations of the smaller multisegmental interneurons were in the main axon fairly near the synaptic zone. Dendritic penetrations are extremely unlikely, because the dendritic trees of these cells consist of a tuft of fine processes (<5 μ) branching ventromedially off the main axon at one point in the caudal third of the ganglion.

These multisegmental tactile interneurons share many of the properties reported for interneurons in general in crayfish. They have broad receptive fields and widely graded EPSPs reflecting a high convergence of primary afferent input. Spikes do not reset the synaptic potentials, so multiple firings from single EPSPs are common. Variable spike shapes are only rarely seen, indicating the presence of branch spikes and multiple spike-initiating zones within a single ganglion. Many interneurons receive inputs from afferents entering in each of several segments and, furthermore, a volley delivered to one segment may lead to a few spikes which arise from an adjacent ganglion as well as the main burst arising from the segment stimulated. Interneurons B and C are not spontaneously active, nor are the other tactile interneurons which excite the lateral giant.

The typical mode of activation of multisegmental tactile interneurons is by monosynaptic, chemical, antifacilitating excitation from primary tactile afferents. Figure 15 illustrates a very common finding. In Fig. 15*A*, a weak second root shock excited tactile afferents which had only a small effect on interneuron C. Increasing the stimulus intensity slightly recruited a tactile afferent recorded in the root monitor which elicited one-for-one an EPSP in the interneuron. At very low stimulus repetition rates (less than 1/min), this response was all-or-none. The figure shows the first, fourth, and seventh responses to stimuli applied once per 2 sec. The EPSP waned to one-third its initial value with repetition of the stimulus, but the shape of the response remained basically unchanged (within noise-level fluctuations and contamination by other small PSPs). The arrival time of the afferent spike was calculated as discussed above. The synaptic delay was constant at about 1.5 msec for repetitive stimulation at low frequency. The potential thus appears to be a unitary, chemically mediated, and strongly antifacilitating EPSP from a tactile afferent onto interneuron C.

Most of the input to multisegmental tactile interneurons consists of such EPSPs. They can be recruited sequentially by increasing the stimulus intensity (Fig. 16), and form a large compound EPSP which reaches threshold a few millivolts below the apparent resting potential. Further evidence for the chemical nature of this excitation (12) is provided in Figs. 17 and 19, which show

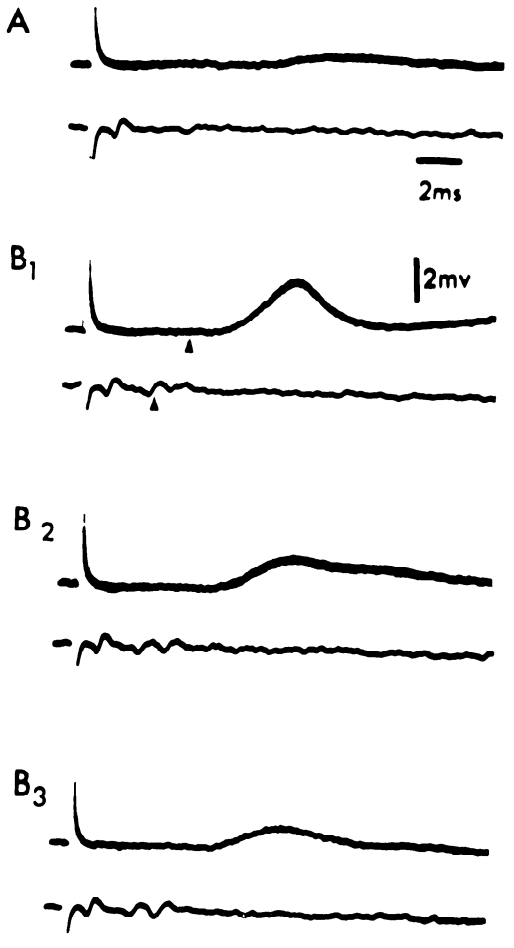

FIG. 15. Properties of monosynaptic afferent EPSPs in tactile interneuron C. *A:* weak stimulation of the second root excites fibers (monitored in the second root on the lower trace) which elicit small depolarizing potentials in the interneuron (upper trace, recorded in the fourth ganglion). *B:* stronger shocks recruit a tactile afferent, whose spike is marked by a triangle, which elicits a large EPSP in the interneuron. The upper triangle indicates the calculated arrival time of the afferent spike. B_1, B_2, and B_3 are the first, fourth, and seventh responses to stimulation at 0.5 Hz. The small late depolarizations are due to occasional activation of tactile afferents whose spikes are not discernible in the root monitor.

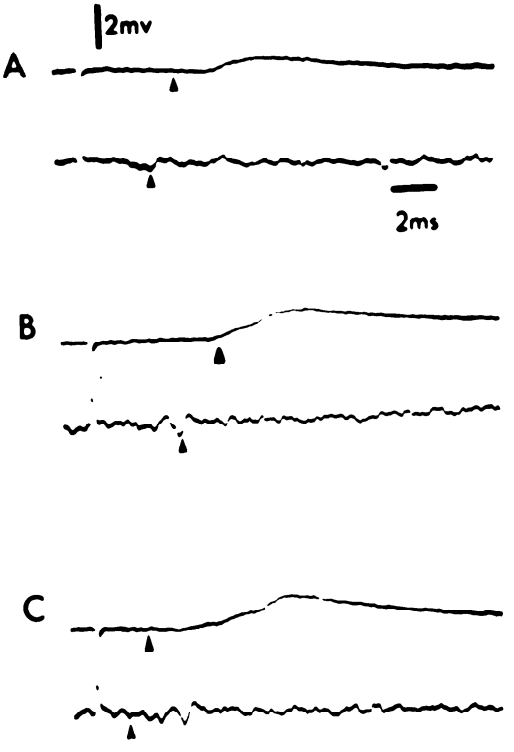

FIG. 16. Unitary EPSPs generated by tactile afferents in interneuron C. The upper trace of each pair records intracellularly from an interneuron process in the third ganglion; the lower trace monitors the afferent volley on the ipsilateral second root to the ganglion. An electrical shock delivered distally to the second root excites a single sensory neuron in *A*, whose spike in the root monitor and arrival time in the ganglion are indicated by triangles. The afferent impulse elicits a delayed EPSP in the interneuron. In *B* and *C*, the stimulus intensity is increased by small increments, recruiting additional afferents indicated on the monitor, each generating an EPSP in interneuron C after a delay of about 1.5 msec.

that the compound EPSP can be enhanced by hyperpolarizing currents and reduced by depolarization.

The susceptibility of synapses to alterations in the concentration of divalent cations has been used as a criterion for distinguishing chemical from electrical synaptic transmission (50). In an attempt to obtain further evidence of the nature of the central synapses discussed here, the effects of altering the ionic media were explored in over 10 experiments.

It has not been possible to maintain a dendritic penetration in an interneuron while changing the bath, so it was necessary to observe either the late response of the lateral giant to root stimulation or the firing rate in interneurons as indirect measures of the effects of ions on the interneuron EPSP. Raising the Mg^{++} concentration to 11 times normal and/or reducing the Ca^{++} concentration to one-third, and maintaining osmolarity at normal, had no effect on the strength of the suprathreshold response of multisegmental tactile interneurons to root stimuli, or to their rate of decline to repetitive

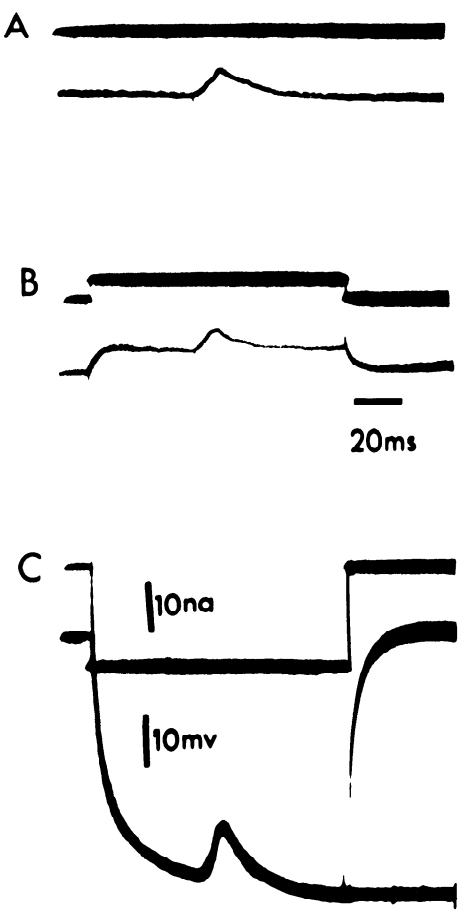

FIG. 17. Effects of postsynaptic polarization on afferent EPSPs in interneuron C. Upper trace: current passed through recording electrode. Lower trace: response of interneuron in third ganglion to second root stimulation of the same segment; bridge balanced to compensate for electrode resistance only.

stimulation. The high Mg^{++}/low Ca^{++} solution did slightly reduce the lateral giant EPSP reversibly. These results were obtained even though the ganglia stimulated were desheathed and 2 hr were allowed for diffusion to occur. I do not take this as evidence that the afferent to interneuron synapse is not chemical, but rather that some diffusion barrier exists between the surface of the ganglion and the synaptic sites buried in dense neuropil. It is unlikely that these chemical synapses are not influenced by Ca^{++} and Mg^{++}, in view of the large number of neuromuscular junctions and neuronal synapses which are so affected (5, 22, 26, 28, 29, 44, 48, 50, 64). This is, however, not the first report of the failure of Mg^{++} and Ca^{++} ions to influence what otherwise appears to be chemical transmission (3, 23, 65). It has also been shown that Ca^{++} deprivation can uncouple electrical synapses (53), which diminishes the usefulness of this procedure for distinguishing chemical from electrical synapses.

Solutions of high Ca^{++} (3 times normal) were also tested for an effect on interneuron responses to peripheral stimulation. If high Ca^{++} raises the thresholds of the interneurons (16, 72), it might be expected to reduce the late components of the lateral giant response selectively (see ref 42, 43). However, the effects of Ca^{++} were too frequently irreversible and, furthermore, Ca^{++} increased the thresholds of the afferent fibers being stimulated as well as affecting the interneurons, making the results meaningless.

Not all of the afferent chemical input to tactile interneurons antifacilitates. For example, some of the input to interneuron A facilitates at moderate frequencies (33). But most of the EPSPs seen in the multisegmental tactile interneurons do antifacilitate to a small fraction of their initial amplitude at low frequencies (73).

LESS COMMON FORMS OF INPUT TO TACTILE INTERNEURONS. Although most of the input to the multisegmental tactile interneurons consists of monosynaptic chemical excitation from afferents synapsing in the same ganglion that they enter, there are other less common forms of activation. For example, maximal afferent stimuli often elicit a small early deflection in the interneuron EPSP showing no synaptic delay, which apparently corresponds to a small electrical component of excitation from some of the afferents. In addition, stimuli applied to an afferent root of an adjacent ganglion often lead to small broad EPSPs, which can summate to produce a spike arising in the monitored segment. It is very difficult to distinguish unitary components of this compound EPSP, so the synaptic delay cannot be estimated. On repetition of the stimulus, these EPSPs decline gradually, and it seems likely that these EPSPs are due to monosynaptic chemical synapses from afferents entering in the adjacent ganglion, as suggested earlier by others (35). It is also sometimes observed that the compound EPSP in an interneuron contains some small fast transients whose latencies vary on repetition of the stimulation (see Fig. 20). It will be shown below that these are generated by polysynaptic pathways. Finally, in three

preparations, inhibition was observed in these interneurons. Figure 18 shows a hyperpolarizing potential recorded from interneuron C on stimulation of the second root of the next posterior (fourth) ganglion. This inhibitory postsynaptic potential (IPSP) remained after the EPSP disappeared after low-frequency repetitive stimulation. The potential was reversed by passing hyperpolarizing current through the electrode. Also, it interacted with an EPSP generated by stimulating the second root of the third ganglion to reduce the amplitude of the latter. This IPSP, difficult to observe against the usual excitatory background, is probably the basis of the inhibitory interactions reported in tactile interneurons earlier (35, 63). The inhibition is probably polysynaptic, since the latency to the IPSP is rather long and variable.

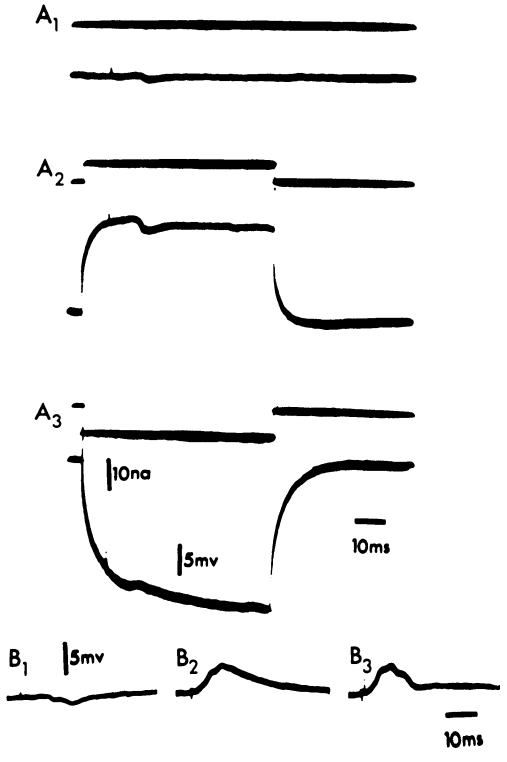

FIG. 18. IPSPs recorded from tactile interneuron C in the third ganglion, in response to stimulation of the second root of the fourth ganglion. *A* shows the effect of postsynaptic polarization on the IPSP; traces as in Fig. 17. *B:* IPSP(B_1) reduced the amplitude of an EPSP elicited by stimulating the second root of the third ganglion (B_2) when the two occurred together (B_3).

In Fig. 19, the amplitudes of the EPSP of Fig. 17 and the IPSP of Fig. 18 are plotted as functions of the change in membrane potential from its resting value produced by passing current through the bridge circuit. The relations can be well approximated by straight lines, and reversal potentials are measured as +40 mv for the EPSP and −6 mv for the IPSP, relative to the resting membrane potential.

Small, fast components with variable latency were observed in the responses of about one-third of the tactile interneurons studied. Some of these may be branch spikes (63), but others are apparently due to synaptic connections between tactile interneurons. Figure 20 illustrates a recording from interneuron B, in which a particular all-or-none component of the response to peripheral stimulation corresponds one-to-one to spikes occurring in interneuron C. The field potential of the spike in interneuron C, which was observed after the microelectrode

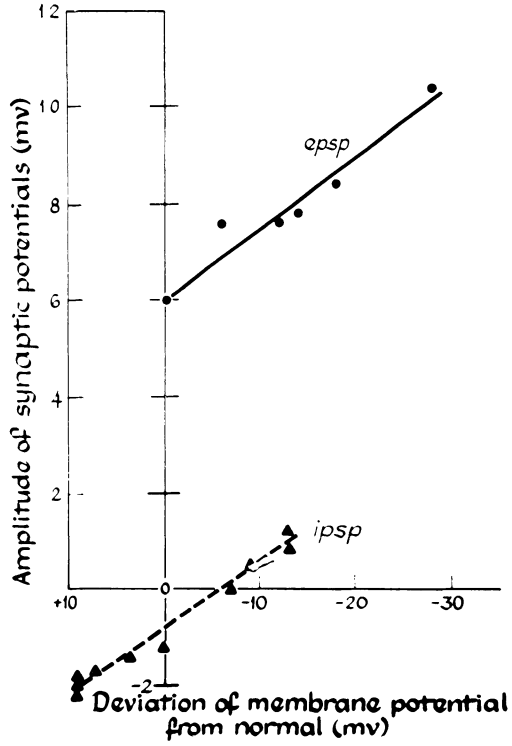

FIG. 19. Relation between the amplitudes of the postsynaptic potentials in interneuron C and the level of polarization. Data from experiments of Figs. 17 and 18.

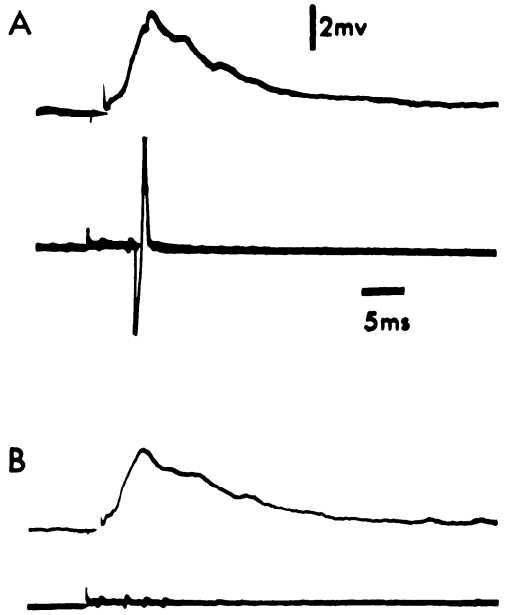

FIG. 20. EPSP in tactile interneuron B associated with impulses in tactile interneuron C. The upper trace is an intracellular recording from cell B in the fourth ganglion. The lower trace monitors the activity in interneuron C in the 3/4 connective. Whenever cell C fires in response to stimulation of the second root of segment four (*A*), the spike is associated with a fast EPSP in cell B. (Spike retouched.)

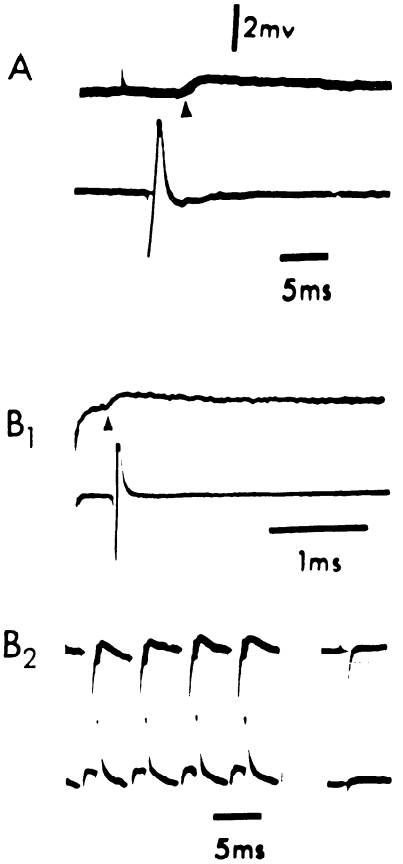

FIG. 21. Properties of excitatory connections between tactile interneurons. Upper trace is an intracellular recording from a tactile interneuron; lower trace monitors cord activity in the 5/6 (*A*) or 3/4 (*B*) connective. *A:* stimulation of interneuron A in the 4/5 connective elicits an electrical EPSP in an unidentified tactile interneuron in the sixth ganglion. B_1: in another preparation, stimulation of A in the 5/6 connective elicits an EPSP in interneuron C in the fourth ganglion. In B_2, the EPSP follows stimulation frequencies up to 200 Hz. The record on the right of B_2 shows the artifacts generated by a subthreshold stimulus. (Spikes retouched in *A* and B_1.)

was withdrawn from interneuron B, was smaller and differently shaped than this potential (cf. Fig. 11). The latter was thus identified as a synaptic potential. Several such connections have been observed, and their properties are illustrated in Fig. 21. In each experiment, one interneuron is isolated from the nerve cord for stimulation while recording from another. Figure 21*A* shows responses from an excitatory connection between interneuron A and interneuron C in the fourth segment. These EPSPs have no synaptic delay and follow high-frequency stimulation of the presynaptic element, and are therefore electrically mediated. The significance of these electrical interneuronal couplings will be treated in a later publication.

Table 1 summarizes the excitatory connections described among the elements involved in generating an escape response to phasic abdominal mechanical stimuli. In addition, the table shows several possible connections which have been looked for and do not exist. Other possibilities exist which have not been explored. For instance, there may be recurrent connections from fast flexor motoneurons onto the tactile interneurons. Such possibilities seem remote, however. Connections to the nongiant fast flexor motoneurons from the giant fibers have been reported by Wiersma (67) and by Kennedy and co-workers (37, 61), and are studied in more detail in the third paper in this series (74).

TABLE 1. *Excitatory connections in third abdominal ganglion among elements in escape response circuitry*

Postsynaptic Neurons	Tactile Afferents	Interneuron A	Interneuron B	Interneuron C	Lateral Giant	Fast Flexor Motoneurons
Interneuron A	C, A, (F)	-	O	O	O	
Interneuron B	C, A, (E, B)	E	-	E	O	
Interneuron C	C, A, (E, B)	E		-	O	
Lateral giant	E	E	E	E	-	O
Fast flexor motoneurons					E, B	-
Fast flexor muscles	O	O	O	O	O	C, F

C = chemical; E = electrical; A = antifacilitating; F = facilitating; O = no effect; B = amplified by branch spikes. Blanks indicate interactions not tested. Parentheses mark less prominent aspects of junctions.

DISCUSSION

Functional anatomy of lateral giant neuron

Certain properties of the potentials generated in the lateral giant could be correlated with the position of the recording microelectrode in the neuron. For example, recordings from the most ventrolateral dendrites always contained the largest and most distinguishable unitary EPSPs. This part of the dendritic tree lies in the field of entrance and divergence of the second and first root afferent axons (30), and also in the region through which many of the tactile interneurons course (see Fig. 8; also ref 69). It seems highly probable that most of the electrical junctions on the lateral giant dendrite are located in this part of the dendritic tree.

The position of the spike-initiating zone can also be inferred from the results. The threshold for initiating orthodromic spikes is between 52 and 57 mv in the dendritic recordings of suprathreshold orthodromic responses illustrated in Fig. 14. However, the same threshold measured in the main axon just rostral to the dendrite is about 7–8 mv (40). These results indicate generally that the latter recording site is much closer to the spike-initiating zone than the former, which in turn is much closer to the major synaptic zone for tactile activation. This interpretation is also borne out by the greater amplitude of the spike recorded from near the proximal lateral giant dendrite (85–100 mv) as compared to those spikes seen from the distal dendrite (40–70 mv).

Organization of input onto lateral giant

In the present study, it has not been possible to demonstrate anything but electrical excitation of the lateral giant in response to tactile stimulation. This input is predominantly polysynaptic, and highly convergent. The experiments eliminate an alternative proposal suggested by Krasne (40) on the basis of preliminary results. He thought that the fast transient unitary all-or-none components of the late response represented branch spikes in the lateral giant's dendritic tree. However, the fact that each such brief depolarization can be associated with an immediately preceding spike in an interneuron known to connect electrically with the giant fiber precludes this hypothesis and confirms the notion that all of these potentials are electrical EPSPs.

One wonders, then, why the input to the giant neuron is organized in this fashion. The answer may lie in the fact that the giant fiber, like so many other large cells (20, 39, 49), has a very high threshold, particularly when measured from the synaptic zone. Chemical EPSPs summate very nonlinearly, especially when they are large (57)

and they approach their equilibrium level only asymptotically. Moreover, chemical EPSPs in tactile interneurons from tactile afferents have a reversal potential of only 40 mv, and especially when corrected for the estimated distance between the recording electrode and synaptic site (see below), the equililbrium potential is below the threshold for lateral giant excitation. Such chemical synapses could never excite spikes in the giant. Highly convergent electrical input from afferents and several repetitively firing interneurons (Fig. 22) seems to be the only possible way to activate the lateral giant. If such huge giant fibers with high thresholds are really necessary for the rapid and coordinated excitation of motoncurons by electrical transmission (see 74), then the circuit used by the crayfish to mediate single escape responses seems the most appropriate.

Many properties of the lateral giant neuron and the escape circuit associated with it bear a striking resemblance to that of the Mauthner neuron in goldfish. Both cells are very large paired premotor neurons which run the full length of the neuraxis. A single spike in either cell elicits a rapid, powerful, coordinated, and highly stereotyped escape behavior in response to a phasic mechanical stimulus (70). Both cells have large dendrites with several specialized synaptic regions receiving input from discrete sources (4, 8). Both neurons are excited by highly convergent monosynaptic and polysynaptic pathways, and the excitation is at least partially electrical (17). The lateral giant and Mauthner cells each receive recurrent collateral inhibition from both members of the pair (19, 59), although the synaptic mechanisms are not identical. Finally, in both neurons, the spike-initiating locus is distant from the excitatory synaptic region. Consequently, both cells have high thresholds when measured from the distal dendrites receiving excitatory input (18).

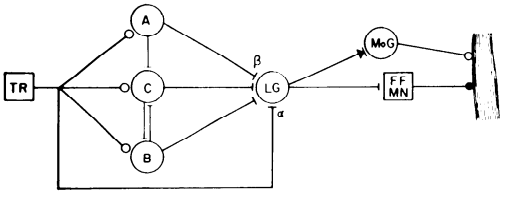

FIG. 22. Schematic circuit of elements and connections concerned with generating a rapid tail flexion to phasic mechanical abdominal disturbances. Large circles represent single neurons; squares encompass populations of similar neurons. Small open circles are antifacilitating chemical synapses or junctions. Filled circles are facilitating neuromuscular junctions. Electrical junctions are shown as bars; the one to the motor giant is rectifying. Fine lines indicate that by and large, multisegmental interneurons are interconnected and are excited by some unisegmental interneurons. The separate pathways generating the α- and β-components of the lateral giant response are indicated. TR: tactile receptors; A, B, C: identified tactile interneurons; LG: lateral giant; MoG: motor giant; FFMN: fast flexor motoncurons. The fast flexor musculature is drawn on the right.

Organization of connections to and among tactile interneurons

The functional anatomy of the tactile interneurons in each ganglion is relatively simple. Kennedy (32) has shown that spikes that arise in any ganglion are initiated within one 35-μ region along the axon. This single spike-initiating zone is probably at the point where the relatively sparse dendritic tree joins the axon (33, 75). Most electrode penetrations are within about 50 μ of this point; I assume an average distance of 25 μ. In the third paper in this series (74), the space constant (λ) of a process 20 μ in diameter was calculated to be 350 μ. The tactile interneurons are about the same size, and presumably have a similar space constant. Thus the electrode is about 0.1 λ from the base of the dendritic tree and the most probable spike-initiating zone. Since synapses are dispersed along the short dendrites, a reasonable estimate for the distance to the center of the synaptic locus is about 0.2 λ. This accounts for the success, at least in some experiments, of finding a reversal potential for the excitatory synapses from the tactile afferents, and for the IPSPs of unknown origin.

The bulk of the input to tactile interneurons is chemical, monosynaptic, afferent, and excitatory. Massive input is not needed to reach the fairly low thresholds of 3–7 mv. Since the electrode is about 0.2 λ from the synapses, the actual site of the EPSP generation would show an EPSP only $e^{0.2}$ or 1.22 times larger than that actually re-

corded by the microelectrode (6); that is, EPSPs up to 10 mv might be generated. This is still a respectably small fraction of the equilibrium potential, which is estimated to be 40 mv degraded by 0.2 λ (6), or about 33 mv. Chemical transmission between afferents and interneurons also provides a convenient point for generating adaptive behavioral habituation. The evidence for ascribing this aspect of escape behavior to this physiological mechanism is presented in detail in the next paper (73).

The input to tactile interneurons from adjacent segments has been described earlier (35). These multisegmental pathways may partially overcome the effect of collision of interneuron spikes initiated in separate ganglia, and thus make the interneuron more responsive to massive stimulation, and more effectively excite the lateral giant. The electrical connections between tactile interneurons may function similarly. Evidence will be presented in a later publication that these junctions actually function to cause clustering of impulses in tactile interneurons. The function of inhibition in these interneurons is at present unknown.

Circuit for one type of escape behavior, and implications for efficient neurophysiological strategies

The circuit of Fig. 22 provides a cascade of electrical input to effectively excite a high-threshold command fiber, allowing for input-specific modifiability of behavior (73), and guaranteeing secure and rapid excitation of a population of motoneurons in executing a stereotyped response (74). The lateral giant sits at the crest of the circuit for escape responses to caudal phasic tactile stimulation. Like many command or decision neurons, its behavioral effect is easily demonstrated by direct stimulation, but its normal route of activation is difficult to discover. If only extracellular techniques are used, stimuli must be chosen carefully to penetrate the neural filters of sensory processors and activate the giant fiber. Given the habituation of the behavior, which results from the properties of the pathways which excite the lateral giant (73), the sensory activation is nearly always rendered subthreshold by the drastic handling involved in dissection. Hence physiological experiments must be performed rapidly on a dissected and gradually deteriorating preparation, which is still recovering from a strongly habituated state. It is not surprising that command fibers are often difficult to excite with natural stimuli. These considerations give rise to the advantages of using intracellular techniques to identify the input to command and decision fibers.

In addition, the present findings shed some light on the typical difficulty of eliciting behavioral responses by stimulating sensory interneurons. If the escape response circuit is at all typical, it appears that it is necessary for a minimal fraction of a population of interneurons of a class (an "excitation cluster," ref 21) to be active before any behavioral effects of stimulation will appear. Stimulation of single primary sensory interneurons, even at high frequencies, may be a quite inefficient strategy for discovering the types of behavior in which these interneurons normally participate. Rather, it may be necessary to identify a hypothetical class or cluster of interneurons, and to stimulate a number of them selectively and simultaneously. The anatomical dispersion of the members of a class often renders this approach unfeasible. In that case, as in the present one, it becomes necessary to work with the next higher hierarchical tier of neurons whose activity effects are describable, and to study the subthreshold effects of members of the lower class of interneurons on the higher ones.

SUMMARY

The afferent limb of the neural circuit mediating escape response in crayfish is described. When a phasic mechanical stimulus is applied to the tail, crayfish frequently evade the disturbance by executing a single rapid tail flexion. This behavior is controlled by the lateral giant fiber, which elicits each tail flip by a single discharge.

The lateral giant is activated by tactile stimulation of the pit hair receptors on the surface of the abdominal carapace. Other sensory systems do not excite this neuron, even subliminally.

By recording intracellularly from the lateral giant dendrite, it is shown that all excitatory input to the lateral giant elicited

by natural tactile stimulation, or by shocking afferent roots, is electrically mediated. Many receptor axons excite the lateral giant directly. However, most of the input is disynaptic, via tactile interneurons. Several tactile interneurons are strongly excited by tactile stimulation and produce large unitary EPSPs in the giant fiber. It is argued that the electrical mode of activation is the most efficient way to excite a large cell with a high voltage threshold.

The tactile interneurons are excited chemically by tactile afferents. Most of the synapses antifacilitate extensively at low frequencies (1/sec to 1/min). Some of the interneurons are electrically coupled.

The full neural circuit comprises receptors, uni- and multisegmental interneurons, a "decision fiber," motoneurons, and muscles. The properties of each junction have been described and related to the stereotyped behavior. Some general strategies for determining the functions and typical routes of activation of central neurons are also suggested.

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REFERENCES

1. BARTH, F. G. A phasic-tonic proprioceptor in the telson of the crayfish *Procambarus clarkii* (Girard). *Z. Vergleich. Physiol.* 48: 181–189, 1964.
2. BENNETT, M. V. L. Physiology of electrotonic junctions. *Ann. N.Y. Acad. Sci.* 137: 509–539, 1966.
3. BIEDEBACH, M., MEUNIER, J.-M., AND TAUC, L. Bases ioniques du potentiel postsynaptique biphasique chez l'*Aplysie*. *J. Physiol., Paris* 60: 220, 1968.
4. BODIAN, D. The structure of the vertebrate synapse. A study of the axon endings on Mauthner's cell and neighboring centers in the goldfish. *J. Comp. Neurol.* 68: 117–160, 1937.
5. BRACHO, H. AND ORKAND, R. K. Effects of calcium on excitatory neuromuscular transmission in the crayfish. *J. Physiol., London* 206: 61–71, 1970.
6. CALVIN, W. H. Dendritic synapses and reversal potentials: theoretical implications of the view from the soma. *Exptl. Neurol.* 24: 248–264, 1969.
7. DAVIS, W. J. Reflex organization in the swimmeret system of the lobster. I. Intrasegmental reflexes. *J. Exptl. Biol.* 51: 547–563, 1969.
8. DIAMOND, J. The activation and distribution of GABA and L-glutamate receptors on goldfish Mauthner neurones: an analysis of dendritic remote inhibition. *J. Physiol., London* 194: 669–723, 1968.
9. ECCLES, J. C. *The Physiology of Synapses*. New York: Academic, 1964.
10. ECKERT, R. O. Reflex relationships of the abdominal stretch receptors of the crayfish. I. Feedback inhibition of the receptors. *J. Cellular Comp. Physiol.* 57: 149–162, 1961.
11. EYZAGUIRRE, C. AND KUFFLER, S. W. Processes of excitation in the dendrites and in the soma of single isolated sensory nerve cells of the lobster and crayfish. *J. Gen. Physiol.* 39: 87–119, 1955.
12. FATT, P. AND KATZ, B. An analysis of the endplate potential recorded with an intra-cellular electrode. *J. Physiol., London* 115: 320–370, 1951.
13. FIELDS, H. L. Proprioceptive control of posture in the crayfish abdomen. *J. Exptl. Biol.* 44: 455–468, 1966.
14. FIELDS, H. L., EVOY, W. H., AND KENNEDY, D. Reflex role played by efferent control of an invertebrate stretch receptor. *J. Neurophysiol.* 30: 859–874, 1967.
15. FRANK, K. AND FUORTES, M. G. F. Stimulation of spinal motoneurones with intracellular electrodes. *J. Physiol., London* 134: 451–470, 1956.
16. FRANKENHAEUSER, B. AND HODGKIN, A. L. The action of calcium on the electrical properties of squid axons. *J. Physiol., London* 137: 218–244, 1957.
17. FURSHPAN, E. J. "Electrical transmission" at an excitatory synapse in a vertebrate brain. *Science* 144: 878–880, 1964.
18. FURSHPAN, E. J. AND FURUKAWA, T. Intracellular and extracellular responses of the several regions of the Mauthner cell of the goldfish. *J. Neurophysiol.* 25: 732–771, 1962.
19. FURUKAWA, T. AND FURSHPAN, E. J. Two inhibitory mechanisms in the Mauthner cell of the goldfish. *J. Neurophysiol.* 26: 140–176, 1963.

20. HENNEMAN, E., SOMJEN, G., AND CARPENTER, D. O. Functional significance of cell size in spinal motoneurons. *J. Neurophysiol.* 28: 560–580, 1965.
21. HORRIDGE, G. A. *Interneurons*. London: Freeman, 1968.
22. HOYLE, G. The effects of some common cations on neuromuscular transmission in insects. *J. Physiol., London* 127: 90–103, 1955.
23. HUGHES, G. M. AND TAUC, L. A unitary biphasic postsynaptic potential (B.P.S.P.) in *Aplysia* brain. *J. Physiol., London* 179: 27–28P, 1965.
24. HUGHES, G. M. AND WIERSMA, C. A. G. Neuronal pathways and synaptic connexions in the abdominal nerve cord of the crayfish. *J. Exptl. Biol.* 37: 291–301, 1960.
25. KAO, C. Y. Postsynaptic electrogenesis in septate giant axons. II. Comparison of medial and lateral giant axons of crayfish. *J. Neurophysiol.* 23: 618–635, 1960.
26. KATZ, B. AND MILEDI, R. A study of spontaneous miniature potentials in spinal motoneurons. *J. Physiol., London* 168: 389–422, 1963.
27. KATZ, B. AND MILEDI, R. The measurement of synaptic delay, and the time course of acetylcholine release at the neuromuscular junction. *Proc. Roy. Soc., London, Ser. B* 161: 483–495, 1965.
28. KATZ, B. AND MILEDI, R. The effect of calcium on acetylcholine release from motor nerve terminals. *Proc. Roy. Soc., London, Ser. B.* 161: 496–503, 1965.
29. KEHOE, J. Single presynaptic neurone mediates a two component postsynaptic inhibition. *Nature* 221: 866–868, 1969.
30. KENDIG, J. J. Structure and function in the third abdominal ganglion of the crayfish *Procambarus clarkii* (Girard). *J. Exptl. Zool.* 164: 1–20, 1967.
31. KENNEDY, D. Physiology of photoreceptor neurons in the abdominal nerve cord of the crayfish. *J. Gen. Physiol.* 46: 551–572, 1963.
32. KENNEDY, D. Input and output connections of single arthropod neurons. In: *Physiological and Biochemical Aspects of Nervous Integration*, edited by F. D. Carlson. Englewood Cliffs, N.J.: Prentice-Hall, 1968, p. 285–306.
33. KENNEDY, D. Crayfish interneurons. *Physiologist* 14: 5–30, 1971.
34. KENNEDY, D., EVOY, W. H., AND FIELDS, H. L. The unit basis of some crustacean reflexes. *Symp. Soc. Exptl. Biol.* 20: 75–109, 1966.
35. KENNEDY, D. AND MELLON, DEF., JR. Synaptic activation and receptive fields in crayfish interneurons. *Comp. Biochem. Physiol.* 13: 275–300, 1964.
36. KENNEDY, D. AND PRESTON, J. B. Activity patterns of interneurons in the caudal ganglion of the crayfish. *J. Gen. Physiol.* 43: 655–670, 1960.
37. KENNEDY, D., SELVERSTON, A. I., AND REMLER, M. P. Analysis of restricted neural networks. *Science* 164: 1488–1496, 1969.
38. KENNEDY, D. AND TAKEDA, K. Reflex control of abdominal flexor muscles in the crayfish. I. The twitch system. *J. Exptl. Biol.* 43: 211–227, 1965.
39. KERNELL, D. Input resistance, electrical excitability, and size of ventral horn cells in cat spinal cord. *Science* 152: 1637–1640, 1966.
40. KRASNE, F. B. Excitation and habituation of the crayfish escape reflex: the depolarization response in lateral giant fibers of the isolated abdomen. *J. Exptl. Biol.* 50: 29–46, 1969.
41. KRASNE, F. B. AND WOODSMALL, K. S. Waning of the crayfish escape response as a result of repeated stimulation. *Animal Behavior* 17: 416–424, 1969.
42. KUPFERMANN, I., CASTELLUCCI, V., PINSKER, H., AND KANDEL, E. Neuronal correlates of habituation and dishabituation of the gill-withdrawal reflex in *Aplysia*. *Science* 167: 1743–1745, 1970.
43. KUPFERMANN, I. AND KANDEL, E. R. Neural controls of a behavioral response mediated by the abdominal ganglion of *Aplysia*. *Science* 164: 847–850, 1969.
44. KUSANO, K. AND HAGIWARA, S. On the integrative synaptic potentials of *Onchidium* nerve cell. *Japan. J. Physiol.* 11: 96–101, 1961.
45. LARIMER, J. L. AND KENNEDY, D. Innervation patterns of fast and slow muscle in the uropods of crayfish. *J. Exptl. Biol.* 51: 119–133, 1969.
46. LARIMER, J., EGGLESTON, A., MASUKAWA, L., AND KENNEDY, D. The different connections and motor outputs of lateral and medial giant fibers in the crayfish. *J. Exptl. Biol.* 54: 391–402, 1971.
47. MELLON, DEF., JR. Electrical responses from dually innervated tactile receptors on the thorax of the crayfish. *J. Exptl. Biol.* 40: 137–148, 1963.
48. MILEDI, R. AND SLATER, C. R. The action of calcium on neuronal synapses in the squid. *J. Physiol., London* 184: 473–498, 1966.
49. NAKAJIMA, S. AND ONODERA, K. Membrane properties of the stretch receptor neurones of the crayfish with particular reference to mechanisms of sensory adaptation. *J. Physiol., London* 200: 165–185, 1969.
50. NICHOLLS, J. G. AND PURVES, D. Monosynaptic chemical and electrical connexions between sensory and motor cells in the central nervous system of the leech. *J. Physiol., London* 209: 647–667, 1970.
51. OTSUKA, M., KRAVITZ, E. A., AND POTTER, D. D. Physiological and chemical architecture of a lobster ganglion with particular reference to gamma-aminobutyrate and glutarate. *J. Neurophysiol.* 30: 725–752, 1967.
52. PABST, H. AND KENNEDY, D. Cutaneous mechanoreceptors influencing motor output in the crayfish abdomen. *Z. Vergleich. Physiol.* 57: 190–208, 1967.
53. PENN, R. D. AND LOEWENSTEIN, W. R. Uncoupling of a nerve cell membrane junction by calcium-ion removal. *Science* 151: 88–89, 1966.
54. PENNAK, R. W. *Fresh-water Invertebrates of the United States*. New York: Ronald, 1953.
55. PILGRIM, R. L. C. AND WIERSMA, C. A. G. Observations on the skeleton and somatic musculature of the abdomen and thorax of *Procam-*

barus clarkii (Girard), with notes on the thorax of *Panulirus interruptus* (Randall) and *Astacus*. *J. Morphol.* 113: 453–487, 1963.

56. PRESTON, J. B. AND KENNEDY, D. Integrative synaptic mechanisms in the caudal ganglion of the crayfish. *J. Gen. Physiol.* 43: 671–681, 1960.
57. RALL, W. Theoretical significance of dendritic trees for neuronal input-output relations. In: *Neural Theory and Modeling*, edited by R. F. Reiss. Stanford: Stanford Univ. Press, 1964, p. 73–97.
58. REMLER, M., SELVERSTON, A., AND KENNEDY, D. Lateral giant fibers of crayfish: location of somata by dye injection. *Science* 162: 281–283, 1968.
59. ROBERTS, A. Recurrent inhibition in the giant fibre-system of the crayfish and its effect on the excitability of the escape response. *J. Exptl. Biol.* 48: 545–567, 1968.
60. SCHRAMECK, J. E. Crayfish swimming: alternating motor output and giant fiber activity. *Science* 169: 698–700, 1970.
61. SELVERSTON, A. I. AND KENNEDY, D. Structure and function of identified nerve cells in the crayfish. *Endeavour* 28: 107–113, 1969.
62. SHERRINGTON, C. S. Some functional problems attaching to convergence. *Proc. Roy. Soc., London, Ser. B* 105: 332–362, 1929.
63. TAKEDA, K. AND KENNEDY, D. The mechanism of discharge pattern formation in crayfish interneurons. *J. Gen. Physiol.* 48: 435–453, 1965.
64. TAUC, L. Transmission in invertebrate and vertebrate ganglia. *Physiol. Rev.* 47: 521–593, 1967.
65. TAUC, L., EPSTEIN, R., AND MALLART, A. Action des ions Mg^{++} et Ca^{++} sur les potentiels postsynaptiques unitaires chez l'*Aplysie*. *J. Physiol., Paris* 57: 284, 1965.
66. VAN HARREVELD, A. A physiological solution for freshwater crustaceans. *Proc. Soc. Exptl. Biol. Med.* 34: 428–432, 1936.
67. WIERSMA, C. A. G. Giant nerve fiber systems of crayfish. A contribution to comparative physiology of synapse. *J. Neurophysiol.* 10: 23–38, 1947.
68. WIERSMA, C. A. G. AND BUSH, B. M. H. Functional neuronal connections between the thoracic and abdominal cords of the crayfish, *Procambarus clarkii* (Girard). *J. Comp. Neurol.* 121: 207–235, 1963.
69. WIERSMA, C. A. G. AND HUGHES, G. M. On the functional anatomy of neuronal units in the abdominal cord of the crayfish, *Procambarus clarkii* (Girard). *J. Comp. Neurol.* 116: 209–228, 1961.
70. WILSON, D. M. Function of giant Mauthner's neurons in the lungfish. *Science* 129: 841–842, 1959.
71. WINE, J. J. AND KRASNE, F. B. The organization of escape behaviour in the crayfish. *J. Exptl. Biol.* 56: 1–18, 1972.
72. WRIGHT, E. B. AND TOMITA, T. A study of the crustacean axon repetitive response. II. The effect of cations, sodium, calcium (magnesium), potassium and hydrogen (pH) in the external medium. *J. Cellular Comp. Physiol.* 65: 211–228, 1965.
73. ZUCKER, R. S. Crayfish escape behavior and central synapses. II. Physiological mechanisms underlying behavioral habituation. *J. Neurophysiol.* 35: 621–637, 1972.
74. ZUCKER, R. S. Crayfish escape behavior and central synapses. III. Electrical junctions and dendrite spikes in fast flexor motoneurons. *J. Neurophysiol.* 35: 638–651, 1972.
75. ZUCKER, R. S., KENNEDY, D., AND SELVERSTON, A. I. Neuronal circuit mediating escape responses in crayfish. *Science* 173: 645–650, 1971.