7 Escape Behavior in Crayfish

Anywhere you go in the animal kingdom, there is always someone who wants to ruin your day. You may be the victim of an aggressive attack by an animal with a serious attitude over territory or a mate, another predator may have you in mind as a meal, or the attacking animal might just be having a bad day. Whatever the cause, this kind of scenario usually necessitates some sort of escape response, and from an adaptive point of view, the sooner the better.

The animal we consider in this chapter, the crayfish, is a highly accomplished escape artist that uses an extremely rapid and powerful flexion of its abdomen to thrust itself through the water to get out of harm's way. This striking behavioral maneuver is called a tail flip and is triggered in response to a sudden tactile stimulus. The tail flip occurs very quickly, with movements beginning less than two-hundredths of a second after a stimulus. Moreover, it is quite complex, involving the coordination in both time and space of many diverse muscle groups in many abdominal segments. Finally, the expression of the escape response is decisive; it cancels all other behaviors that could interfere with its execution, and then releases them from inhibition to permit subsequent adaptive behaviors such as reextension of the abdomen and swimming away. Thus the crayfish tail flip provides the neurobiologist with a superb opportunity to identify in cellular terms the organizational principles that permit the expression of a complex and highly integrated motor response.

Crayfish belong to the order Decapoda, in which there are three families; two (Astacidae and Cambaridae) are found in the Northern Hemisphere and the third (Parastacidae) in the Southern Hemisphere. Crayfish are the largest macroinvertebrates in temperate freshwater ecosystems; all the families occupy a wide range of habitats. The species most widely used in neurobiological studies is *Procambarus clarkii*, sometimes called the red swamp crayfish. This species is among those known as secondary burrowers; they are often found in areas flooded seasonally, but they remain in their burrows at other times. Thus these animals are well adapted to so-called lentic systems, which are periodically flooded but are dry in the summer.

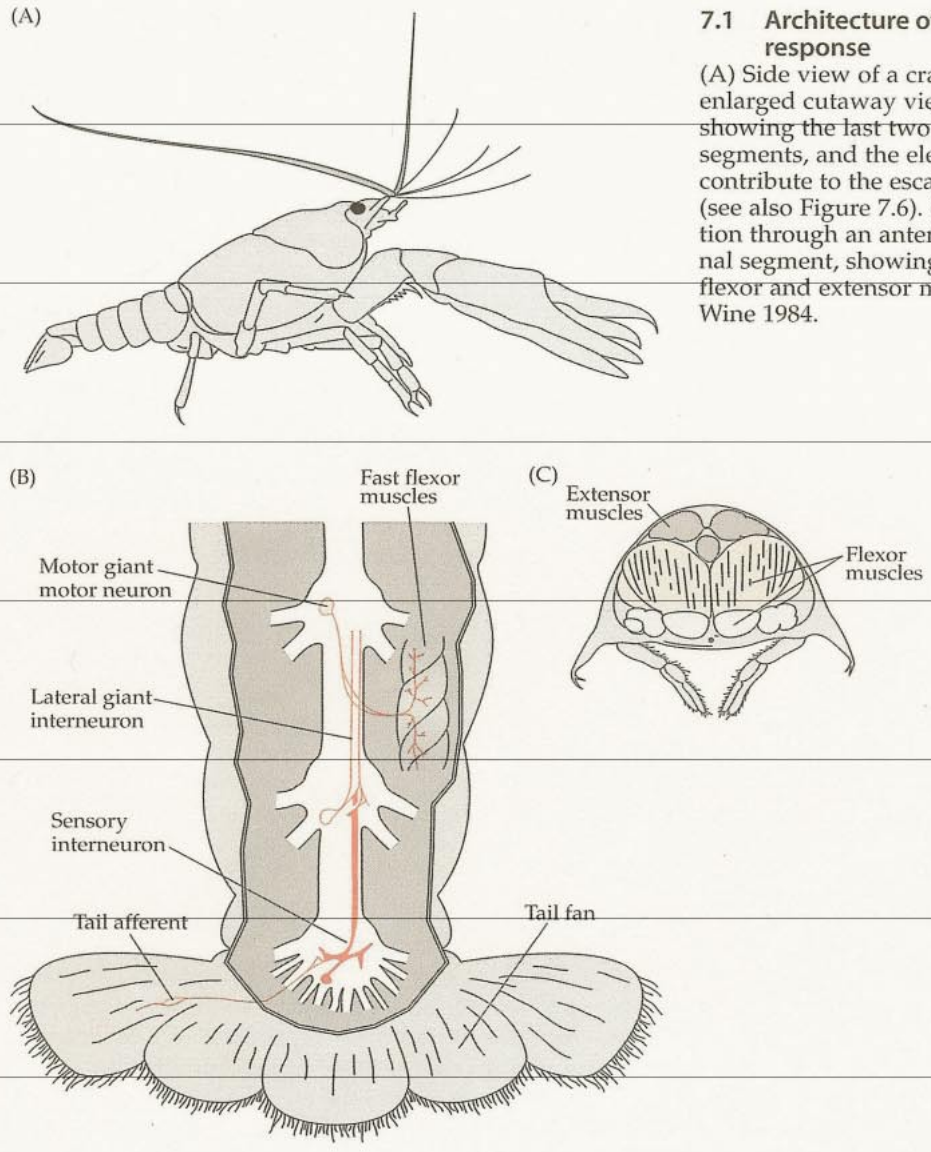
The diet of most crayfish consists largely of plant detritus, the nutritional value of which is derived from the epiphytic fungal and bacterial organisms involved in the decomposition process. This type of diet is often supplemented with small invertebrates and fish, as well as other crayfish. Although food of animal origin often constitutes the smallest portion of the diet, it is important for providing the animals with essential organic compounds.

Crayfish have a host of predators both on land and in the water, including dragonfly nymphs, fish (such as catfish and trout), birds (especially wading birds such as egrets and herons), bullfrogs and eels, and many mammals, such as raccoons, opossums, and others (including humans). With all these animals in hot pursuit, it is clear why the crayfish has evolved a highly effective escape response.

Behavioral Features and Functional Anatomy of the Escape Response

In response to a tactile stimulus delivered to its body, the crayfish exhibits different types of escape responses, depending in large part on the site and duration of the stimulus. All the responses involve powerful abdominal flexions that propel the animal through the water. Before we describe the details of these different escape responses, a brief overview of crayfish anatomy is warranted.

The crayfish, like other decapod crustaceans, has a hard external skeleton (the carapace) in the thoracic region, from which extend its walking legs and ominous-looking front claws (Figure 7.1A). The posterior portion of the animal is made up of abdominal segments that are flexibly interconnected to form a functional appendage, and for this reason it is often called a **tail**. The abdomen has five similar segments and two terminal segments that have been specialized to form a **tail fan** (Figure 7.1B), the enlarged surface of which can act like the blade of a paddle. Each of the five anterior segments contains a massive set of phasic axial **flexor** and **extensor muscles** (Figure 7.1C). The crayfish has two distinct forms of locomotion, both of which use the abdomen. The vast majority of the time locomotion is accomplished by walking, during which the abdomen is held extended (Figure 7.1A) and used for balance and steering.



7.1 Architecture of the escape response
(A) Side view of a crayfish. (B) An enlarged cutaway view of the tail, showing the last two abdominal segments, and the elements that contribute to the escape response (see also Figure 7.6). (C) Cross section through an anterior abdominal segment, showing the phasic flexor and extensor muscles. After Wine 1984.

However, the abdomen is always cocked and ready to be called into action for an escape response, the **tail flip**. Extensive behavioral and cellular analyses of the crayfish tail flip response have been carried out by Franklin Krasne, Jeffrey Wine, and their colleagues. In behavioral studies these coworkers have described the fact that tail flip responses in the crayfish have three basic forms, each one named after the neural circuitry that contributes significantly to its

expression. As we shall discuss later in this chapter, crayfish possess two pairs of giant axons that traverse the entire length of the nervous system. These axons arise from two pairs of "giant" interneurons, the **lateral giant interneurons (LGIs)** and the **medial giant interneurons (MGIs)**, each of which plays a critical role in the production of a particular kind of rapid tail flip. In addition, a third form of tail flip is produced by circuitry not involving the LGIs or MGIs, called the nongiant escape circuitry; thus this tail flip is called a **nongiant** escape response. The exact form and sequence of all three types of tail flip responses have been characterized by Wine and Krasne using high-speed cinematography. A summary of their results follows:

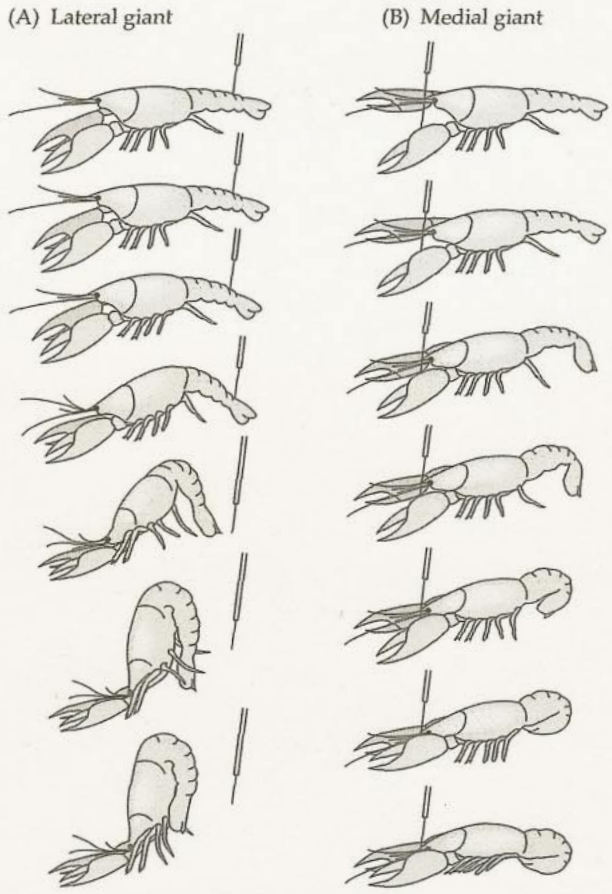
Lateral giant escape. A very abrupt tactile stimulus to the abdomen or tail fan evokes a short-latency (about 10 ms), highly stereotyped single tail flip that rotates the rear end of the animal upward and forward, forming essentially the first half of a somersault (Figure 7.2A). This complex maneuver moves the animal up and away from the eliciting stimulus.

Medial giant escape. These responses are triggered by a tactile (or visual) stimulus with a very abrupt onset delivered to the front of the animal. They are characterized by a rapid tail flip that propels the animal directly backward (Figure 7.2B).

Nongiant escape. These responses can be elicited by many different kinds of tactile disturbances; the eliciting stimuli need not be so abrupt as those for lateral and medial giant escape. The nongiant responses are so named because they do not involve the actions of either the LGIs or the MGIs. They are typically of long latency (80–500 ms) and most often occur in swimming sequences that are flexible in form, permitting visually guided steering.

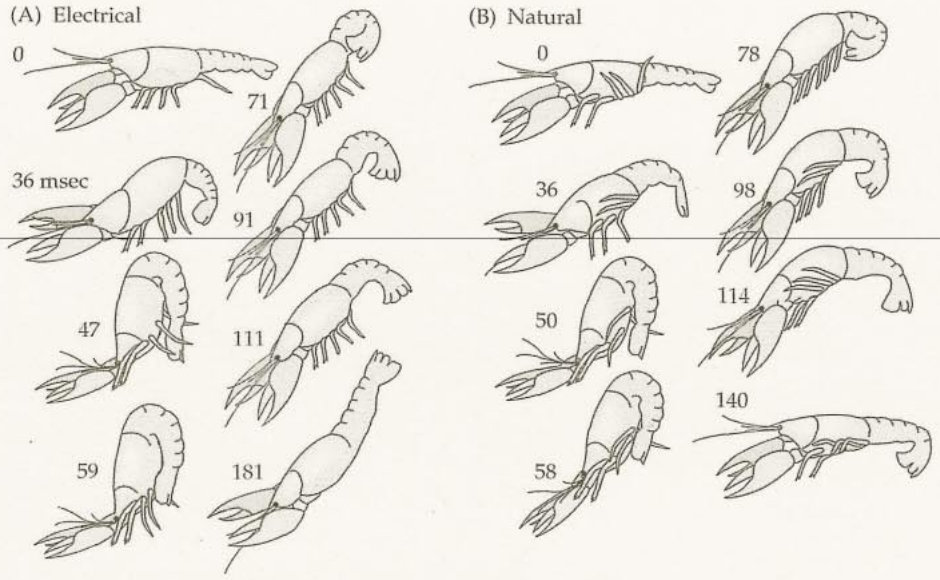
Stimuli that trigger giant-mediated tail flips usually also trigger subsequent episodes of nongiant swimming. Thus the combined actions of the giant and nongiant systems optimize effective escape: The first response is extremely rapid and immediately moves the animal some distance away from the potentially threatening stimulus; however, this rapid response is not particularly directed. The second response phase has a slower expression, but it can produce directed swimming episodes to complete the evasive maneuver. Finally, all three types of flexor reactions are coupled to reextension reactions, which return the abdomen to a biomechanical state in which additional flexor contractions can be used for further evasive action.

As mentioned previously, all flexions and extensions used in tail flip reactions are produced by a specialized set of abdominal flexor and extensor muscles (see Figure 7.1C); these muscles are not used for any purpose other than the flexion and reextension components of the tail flip escape response. The primary differences between the LGI and MGI responses seen in Figure 7.2 can be accounted for by differential contraction patterns of these muscles during the two types of behaviors. During MGI flexions, *all* of the phasic abdominal



7.2 Photographic analysis of tail flips initiated by the giant interneurons
Drawings from high-speed cinematographs. (A) A tactile stimulus to the tail fan at the rear of the animal elicits a tail flip mediated by the lateral giant interneurons that moves the animal upward. (B) The same stimulus to the front of the animal elicits a tail flip mediated by the medial giant interneurons that propels the animal backward. After Wine and Krasne 1972.

flexor muscles contract. Thus the tail fan and rear end of the animal (which normally are slightly bent) are rapidly drawn forward underneath the animal by the flexor actions exerted at more rostral segments. This action causes a strong horizontal thrust with a backward trajectory (see Figure 7.2B). In contrast, during LGI tail flips, flexor muscles of *only the rostral half* of the abdomen contract; the rear end of the animal remains extended and acts as the flat blade of a paddle. The action of this paddle against the surrounding water causes the lift and forward rotation that is observed (see Figure 7.2A). Thus a critical difference between MGI- and LGI-mediated flips is the *omission* of flexion in the caudal (rearward) segments of the abdomen for the LGI response.



7.3 Natural versus electrical stimulation of the tail flip
Electrical stimulation of an MGI elicits a tail flip response very similar (A) to the one elicited by a tactile stimulus to the front of the animal (B). The numbers in parentheses refer to the time (in milliseconds) from the onset of the stimulus. After Wine and Krasne 1982.

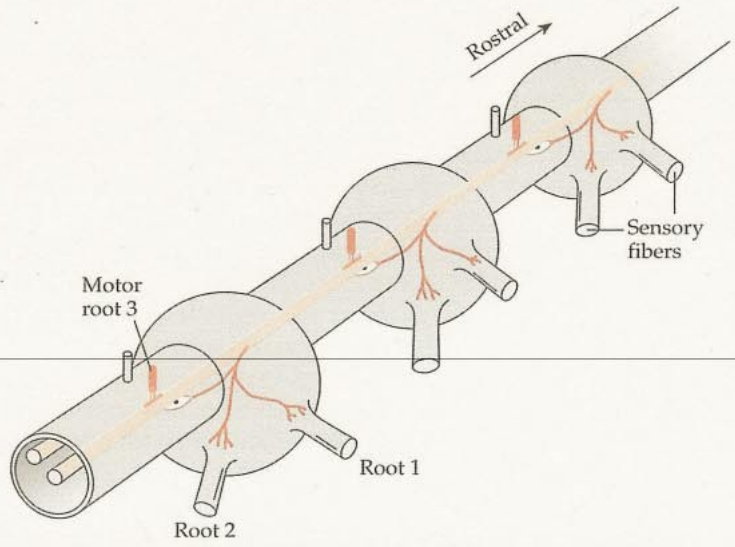
As we shall discuss in detail in this chapter, one of the tremendous assets of the crayfish system is that individual LGI and MGI axons can be activated artificially in freely moving animals by means of brief electrical pulses delivered via implanted electrodes in the nerve cord. This procedure was developed by George Johnson and subsequently extended by the pioneering studies of C. A. G. Wiersma. By this means it was possible to compare tail flips in response to natural events with those in response to direct stimulation of the giant interneurons. An example of this kind of experiment is shown in Figure 7.3, which allows comparison of a tail flip evoked by tapping the front of the animal with one evoked by direct electrical activation of the MGI. The two responses are strikingly similar. Experiments such as this have shown convincingly that both the medial and the lateral giant fiber systems in the crayfish can, by themselves, serve as triggers that initiate the complex sequence of motor events underlying the tail flip.

Neuronal Architecture of the Escape Response

The central nervous system of the crayfish follows a typical invertebrate plan: It is constructed of a series of **ganglia**, and communication between the gan-

glia is mediated by pairs of **connectives**. Each ganglion communicates with the periphery by means of roots that exit the nerve cord bilaterally. The principal components of the CNS are (1) the brain, (2) a single large subesophageal ganglion complex, (3) five thoracic ganglia, and (4) six abdominal ganglia, which contain the neural circuitry for the tail flip. In the abdominal nervous system, each anterior segment is controlled by a single ganglion, which, as already mentioned, communicates with neighboring ganglia via paired connectives, and with the periphery via three paired roots (Figure 7.4). The most anterior root (root 1) is a mixed sensory–motor nerve that innervates fanlike structures called the swimmerets; the second nerve (root 2) is also mixed, containing motor fibers that innervate the extensor muscles; the most caudal nerve (root 3) is purely motor, containing motor fibers to the flexor muscles. The last abdominal ganglion actually results from the fusion of two ganglia; some of the circuitry in this ganglion is specialized to receive input from sensory hairs on the tail fan and to control tail fan movements.

In the abdominal nerve cord two pairs of giant axons stand out from all the rest. These are the lateral and medial giant axons. The medial giants have their cell bodies and dendrites in the brain, where they receive their sensory input; their axons then project down the entire nerve cord to the last abdominal ganglion. The lateral giant (depicted in Figure 7.4) is different. It consists



7.4 The abdominal ganglia contain the circuitry for the tail flip
Sensory and motor fibers enter and exit through roots, which connect the nervous system to the periphery. Ganglia connect to one another by means of fiber tracts called connectives. After Krasne 1976.

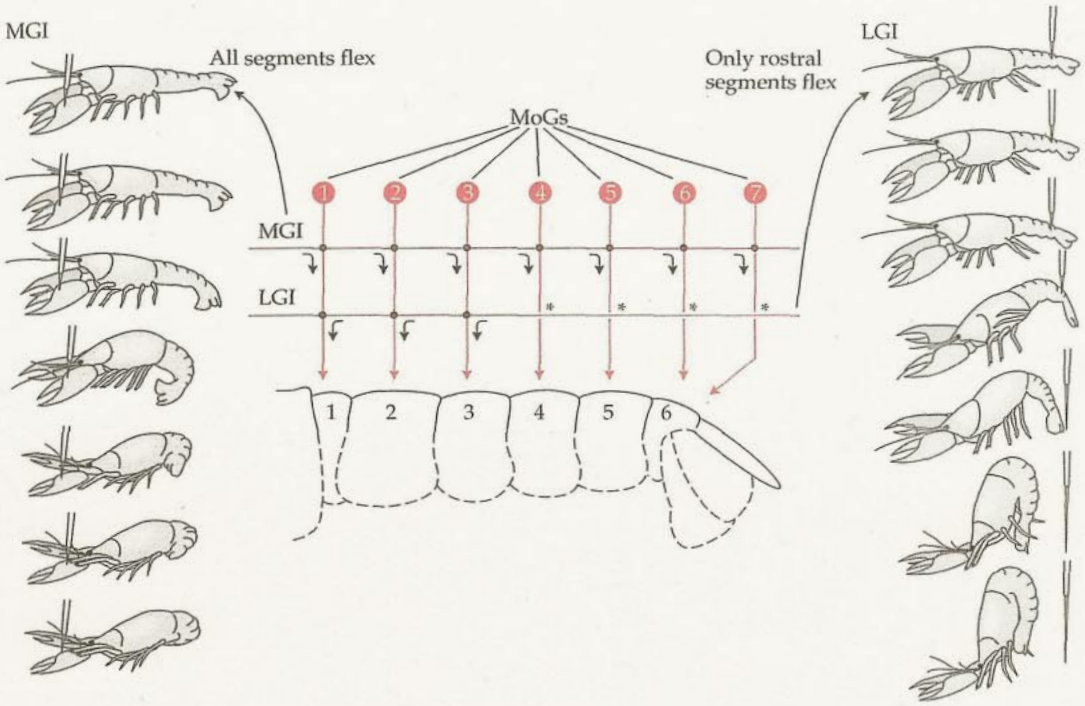
of separate cells that are connected end to end by means of electrical synapses (called **septate**, or **segmental**, **synapses**). Each abdominal segment contains a cell body and dendrites of the lateral giant in that segment; thus the lateral giant receives its sensory input exclusively from the abdominal segments. Each lateral giant interneuron then projects an axon to the next segment, where it forms a segmental synapse with the corresponding lateral giant axon in that segment. With this arrangement the lateral giant operates as if it were a single, continuous giant fiber spanning the abdominal segments.

The neural circuit for the tail flip

We have seen that an abrupt stimulus to the front of the crayfish elicits a pattern of tail flip that is different from that elicited by a comparable stimulus to the rear of the animal (see Figure 7.2). We have also just learned that the MGIs receive their sensory input from the head region, whereas the LGIs receive their input from the rear (abdominal) region. Moreover, direct electrical activation of the MGIs or LGIs produces tail flips comparable to those elicited from anterior or posterior stimuli, respectively (see Figure 7.2). Finally, when one chronically records from the MGIs and the LGIs in freely moving animals, the pattern of tail flips shown in Figure 7.2 is invariably preceded by an action potential in the appropriate giant axon. Thus the MGIs and LGIs are unequivocally implicated in the two types of response patterns.

As we shall discuss, the giant axons have direct, powerful synaptic connections to a specific class of flexor motor neurons called the **motor giants** (**MoGs**), which connect to all the fast flexor muscles in each abdominal segment. Activation of the MoGs thus contributes significantly to the flexion of each segment during the tail flip. By examining the connectivity patterns of the giant axons with the motor giants, we can gain insights into the specific orientation of the initial escape movement generated by each giant fiber. The side panels in Figure 7.5 are the same tail flips shown in Figure 7.2. Tapping the head causes the MGIs to fire, which in turn activates *all* the motor giants, causing *all* segments to flex. The result is the rapid curling movement that propels the animal backward. In contrast, tapping the abdomen causes the LGIs to fire, but they have output only to the MoGs in the rostral segments, which undergo rapid flexion. Since the LGIs have no connection to the MoGs in the caudal segments (missing synapses are indicated by an asterisk in Figure 7.5), those segments remain straight and thus cause the thrust to be directed downward, thus pitching the animal upward as well as forward. In summary, the differential sensory input to the LGIs and MGIs, taken together with their patterns of connections to their target motor neurons, can explain in large measure the different receptive fields, topographies, and trajectories of the two types of tail flip escape responses.

Of the two giant fiber systems, the circuitry for LGI-mediated escape has been far more extensively analyzed. Thus in the remainder of the chapter we will focus primarily on this system.

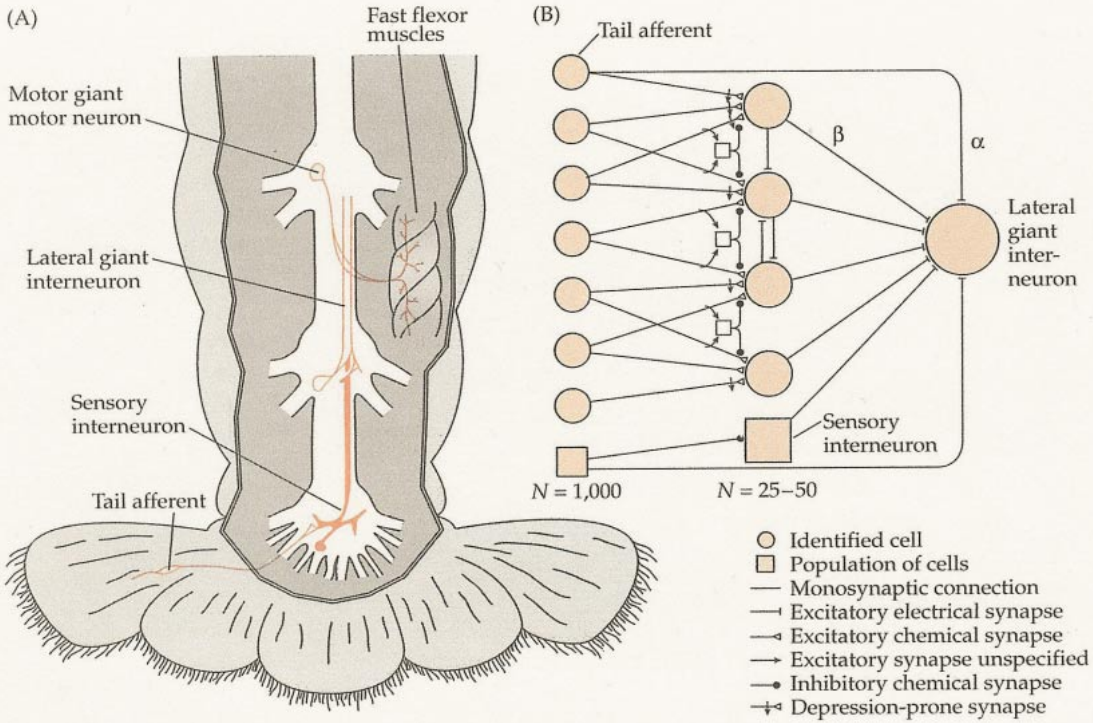


7.5 Connectivity patterns of the giant interneurons
The left and right side panels show MGI- and LGI-mediated tail flips, respectively. The schematic wiring diagram illustrates that the MGIs make synaptic contact (solid circles) with the motor giants (MoGs) in every abdominal ganglion, whereas the LGIs connect with the MoGs only in ganglia 1 through 3. (Asterisks indicate the lack of an LGI synapse in ganglia 4 through 7.) The abdominal segments 1 through 6 are illustrated below. The net result of this connectivity is that the MGIs cause all segments to flex, whereas the LGIs activate only the rostral segments. After Wine and Krasne 1982.

The LGI circuit

In considering the neural circuit for the LGI-mediated tail flip, it is helpful to begin with the central core of the circuit, which is diagrammed in Figure 7.6. There are five basic components of this core circuit:

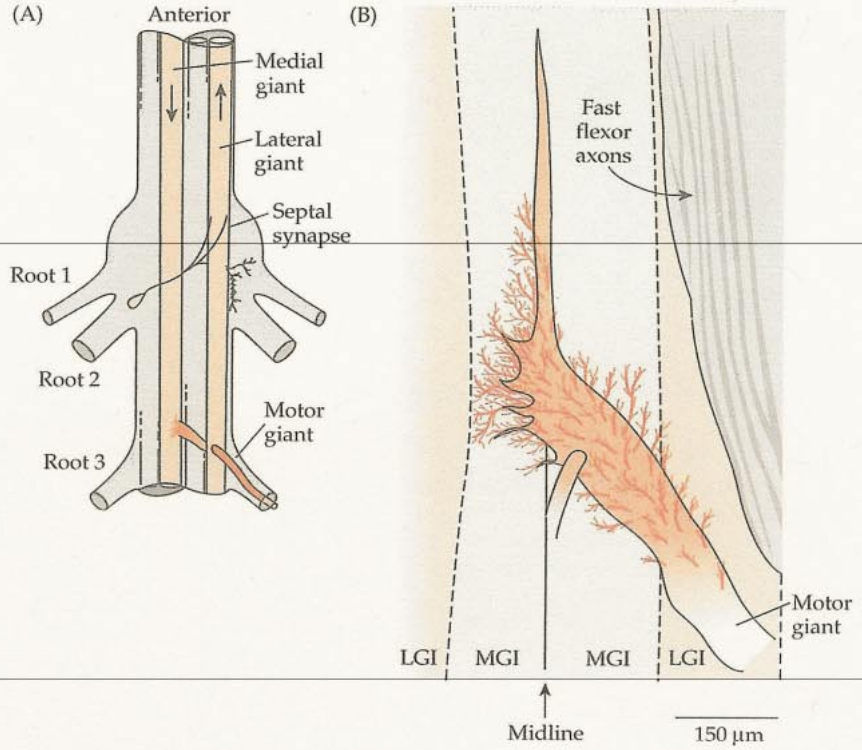
1. **Sensory input.** The major sensory input to the LGI comes from approximately 1000 cuticular hairs (tail afferents) that cover the abdomen; each hair contains bipolar receptors that are directionally sensitive to movement.
2. **Sensory interneurons.** These cells receive direct input from sensory neurons and relay that input to the LGIs. There are two main classes of



7.6 The tail flip circuit
(A) The core LGI circuit (same as in Figure 7.1B). (B) A schematic wiring diagram of the tail flip circuit. The direct inputs to the LGIs via electrical synapses (α) and indirect inputs via chemical synapses (β) are indicated. After Wine and Krasne 1982; data from Krasne 1969, Zucker 1972, and Kennedy, Calabrese, and Wine 1974.

sensory interneurons: A-cells, which are among a class of cells that fire phasically to tail fan input and project up to the brain, providing input to the LGIs in each abdominal ganglion en route, and C-cells, which are among a class of cells that fire tonically to mechanosensory input and project to the LGIs in each abdominal segment (see Figure 7.8).

3. **Lateral giant interneurons (LGIs).** We have discussed these giant fibers already and will consider them in more detail in the discussion that follows.
4. **Motor giants (MoGs).** These powerful flexor motor neurons have the largest cell bodies in each abdominal ganglion. They extend thin processes that then expand near the exit in the third root and give off tufted dendrites through which the LGIs make direct electrical contact (Figure 7.7).

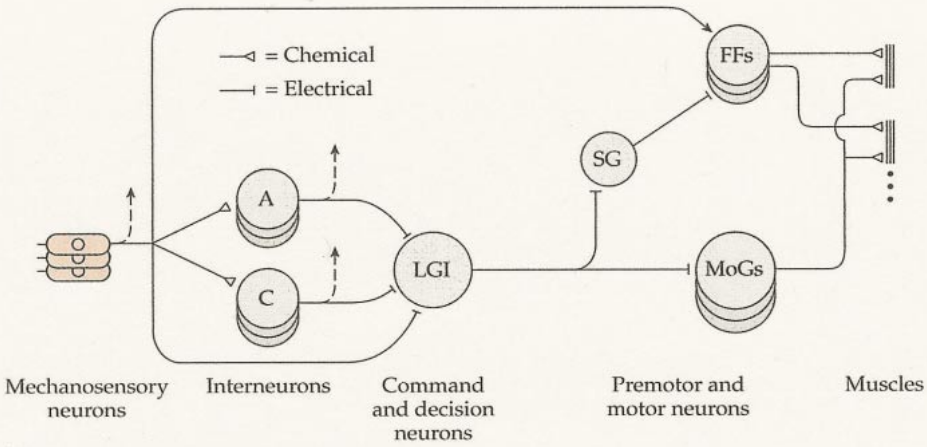


7.7 The motor giant
(A) The position of the MoG as it exits through the third root. (B) Drawing from a photomicrograph showing the connections of the MoG to the MGIs and the LGIs. After Wine 1977.

5. **Fast flexor muscles.** In each segment there are about five phasic flexor muscles, all of which are innervated by the MoG. As we shall discuss, these muscles are also innervated by other excitatory and inhibitory motor neurons.

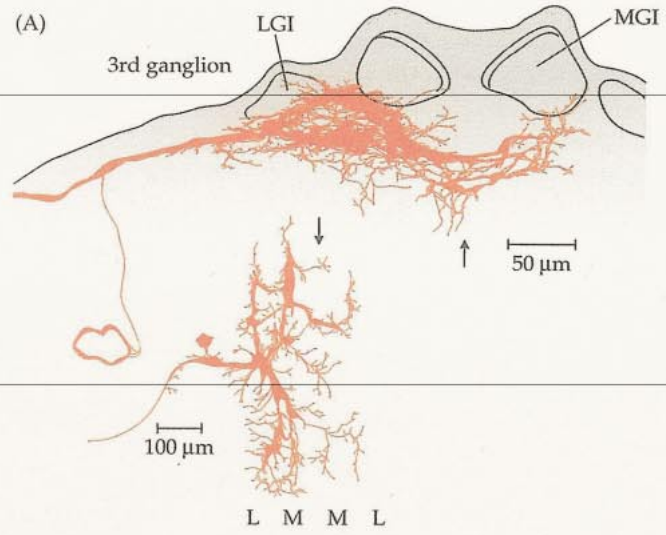
The core circuit shown in Figure 7.6 represents the shortest major pathway from receptors to muscles. Chemical synaptic transmission (which is somewhat slower than electrical transmission, as discussed in Chapter 1) is used only at the input and output stages—that is, between the sensory neurons and the sensory interneurons, and between the MoG and the flexor muscles. All the other connections are electrical, which adds to the processing speed of the circuit.

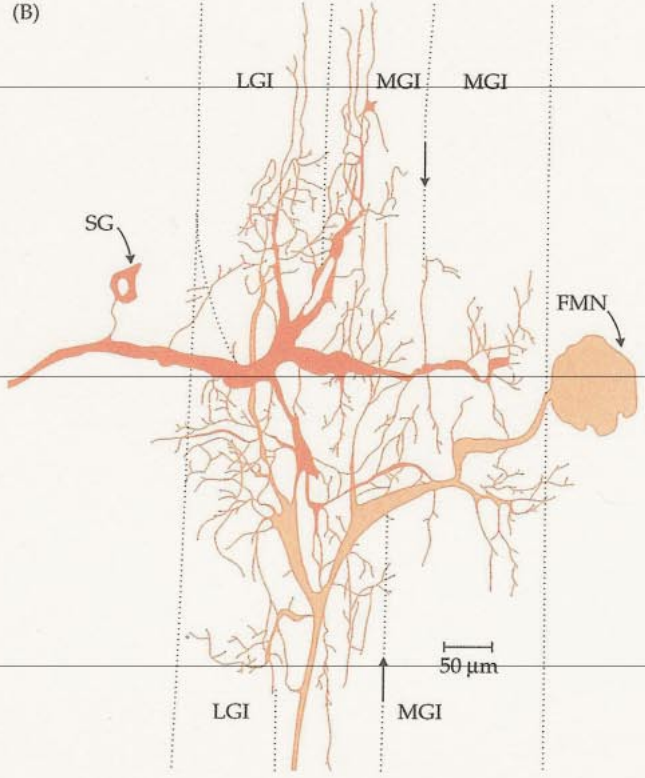
In addition to the core circuit shown in Figure 7.6, a few other elements and connections are important features of the tail flip circuit. These are illustrated in the "wiring diagram" for the tail flip circuit shown in Figure 7.8. The first point to emphasize is that the sensory neurons make contact with the LGIs



7.8 Wiring diagram of the tail flip circuit
This diagram expands on the simpler version shown in Figure 7.6B. A = A-type sensory interneuron, C = C-type sensory interneuron, FF = fast flexor muscle, MoG = motor giant, SG = segmental giant. The dashed lines from mechanosensory neurons and A and C sensory interneurons indicate projections to LGIs in other abdominal segments. After Krasne and Wine 1987.

both *indirectly* (through the A-type and C-type sensory interneurons) and *directly* (through an electrical synapse). When one records intracellularly from the LGI and activates the sensory neurons, it is usually possible to distinguish two short-latency components of the excitatory synaptic input, an **alpha component** reflecting the direct sensory-to-LGI connection, and a **beta component** reflecting the slightly delayed input to the LGIs arriving through the sensory interneurons (see Figure 7.10). In addition, as indicated by the solid line and arrow in Figure 7.8, the sensory neurons can influence the fast flexor motor neurons in a "feed-forward" manner that bypasses the LGI; this sensory influence will be discussed later in the chapter. A second feature of the wiring diagram in Figure 7.8 is that the LGIs can activate the fast flexor muscles by two routes. The first we have discussed, by its direct excitation of the MoGs (see Figures 7.6 and 7.7). The second route is via an intervening premotor interneuron called the **segmental giant (SG)** (see Figure 7.9), which receives an electrical connection from the LGI and then makes electrical synapses onto several fast flexor motor neurons (see Figure 7.8). Each fast flexor motor neuron in turn connects to a few flexor muscles within a segment. Thus the fast flexor motor neurons are arranged in parallel with the motor giant. There is one SG on each side of each abdominal ganglion (the SGs are called giants because of their exceptionally large dendritic trees) (Figure 7.9). They are coupled to the LGIs by an unusually effective electrical synapse that allows the LGI to bring





7.9 The segmental giant
(A) The SG has been injected with a dye that reveals its overall morphology (the cell is shown in color). Top shows cross sections; bottom shows horizontal views.
(B) A higher-power, horizontal view of the SG as it comes into contact with an FF motor neuron (FMN) in the abdominal ganglion (the SG and FMN were injected with different dyes so their anatomical relationship could be seen). FMN = fast flexor motor neuron. After Roberts et al. 1982.

the SGs to firing within a fraction of a millisecond. The net result is an extremely rapid transfer of excitation from the LGIs to the fast flexor motor neurons.

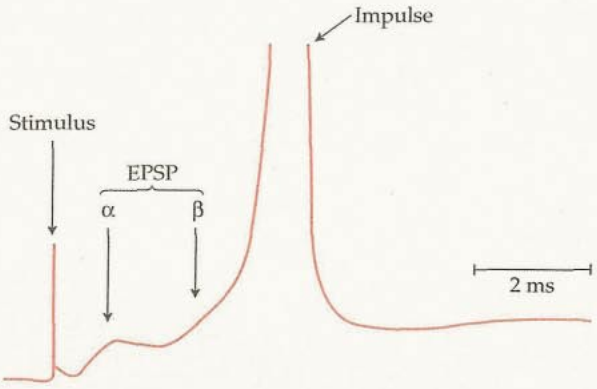
Upon inspection, the wiring diagram shown in Figure 7.8 clearly shows that the LGIs lie at the heart of the circuit: Extensive *convergence* of sensory input is funneled to the LGI, and extensive *divergence* of motor output emanates from the LGI. Thus this giant interneuron occupies an ideal location in the circuit to serve as a "decision switch," or trigger, for the tail flip. Having identified the basic neural circuit for the tail flip, we can now examine how the circuit operates when the animal receives a sudden tap on its tail fan and within a few milliseconds is well on its way to escape.

Flipping out

The escape response mediated by the LGIs has three basic components: (1) rapid flexion of the abdomen, followed by (2) reextension of the abdomen and, finally, (3) swimming. Each of these behavioral components is a relatively independent module of behavior; the LGIs are directly involved only in generating the first component, rapid flexion. As we shall discuss, the initiation of postflexion reextension and swimming involves a chain reflex and delayed sensory activation, respectively.

FLEXION. By far the best stimulus for eliciting a tail flip mediated by the LGIs is an abrupt tap to the abdomen or tail fan. This type of stimulus activates cuticular hair receptors that cover the abdomen. As already discussed, these hair receptors communicate with the LGIs in two ways: (1) directly by means of electrical connections and (2) disynaptically via sensory interneurons to the LGIs (see Figures 7.6 and 7.8). The main difference between these two routes of activation is that the afferent connection onto the sensory interneurons is by means of a chemical synapse. This input constitutes the main synaptic drive to the LGIs. When one records intracellularly from the LGI, it is possible to discern both sources of afferent input. Following stimulation of the hair receptors on the abdomen, a biphasic (compound) excitatory postsynaptic potential (EPSP) is seen in the LGI (Figure 7.10). The first component (the alpha component) reflects summating electrical synaptic input directly from the receptors to the LGIs; the second (the beta component) reflects the input from the sensory interneurons. These two inputs summate in turn to initiate an action potential in the LGI.

As shown by Robert Zucker, several features of the afferent input to the LGIs account for the preferential activation of rapid flexion in response to abrupt stimuli: (1) The chemical synapses between the mechanoreceptors and the sensory interneurons show rapid depression when activated repeatedly (see Figure 7.6B). Thus gradual or prolonged stimuli would not be faithfully transmitted at these synapses. (2) Sensory and interneuronal synapses onto the LGIs are electrical, and the LGIs have a high activation threshold and a short time constant; thus activation of the LGIs requires that the inputs carrying sensory information be almost synchronous to give rise to effective temporal summa-



7.10 Input to the LGI
The LGI receives input from both electrical and chemical synapses, as this intracellular recording shows. Activation of the tail afferents (stimulus) gives rise to two components of the complex synaptic input (EPSP) to the LGI: the short-latency alpha (α) component (electrical input), and the longer-latency beta (β) component (chemical input). The net synaptic input produces an action potential (impulse) in the LGI. (The top of the impulse has been cropped in this illustration.) After Krasne 1969.

tion. Finally, (3) as shown by Donald Kennedy and his colleagues, sensory input evokes recurrent, presynaptic inhibition onto the afferent terminals (see Figure 7.6), thereby effectively creating a narrow time window during which afferent information can access the LGIs.

When sensory input triggers a spike in the LGIs (as shown in Figure 7.10), they activate output to the fast flexor muscles in two ways. The first pathway is by powerful electrical excitation of the motor giants (MoGs), which in turn directly excite the fast flexor muscles (see Figures 7.6 and 7.8). The MoGs are activated exclusively by the giant axons (of the LGIs and MGIs) and appear to be restricted in function to mediation of the giant-mediated tail flips. The second route of excitation to the fast flexor muscles is through the segmental giant (SG), with which the LGIs make electrical synapses. The electrical coupling between the LGIs and SGs is suprathreshold for SG activation; thus this second pathway introduces little transmission delay in the LGI-mediated tail flip.

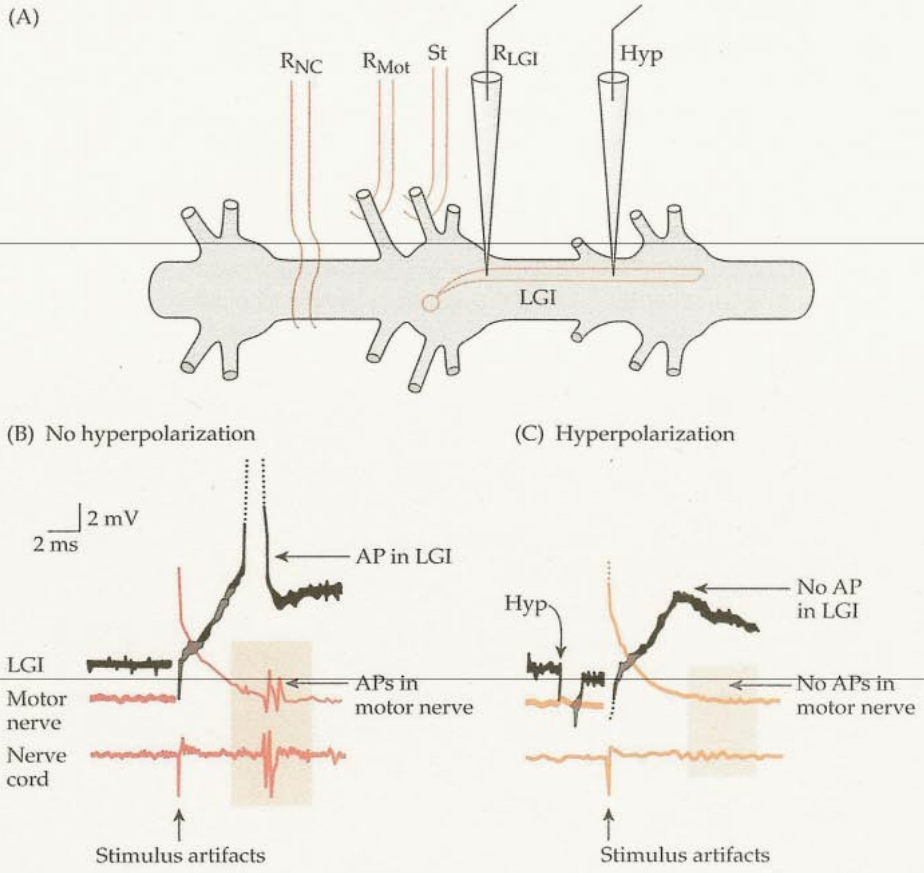
The synaptic actions described here represent the core of the tail flip circuit, and at the heart of the circuit are the LGIs. The remarkable speed of this behavior is attributable to two features of the LGIs: First, they have a very large diameter axon and thus have a very rapid conduction velocity (see Chapter 1); second, every known LGI synapse is electrical, which is a more rapid means of transmission than via chemical synapses. Thus the LGIs appear to act as "decision units" that are critical for the initiation of a tail flip (another term for this type of decision-like function is "command neuron," a concept that we will explore in more detail a little later).

However, even though the LGI is perfectly positioned to act as a critical neuron where the "decision" to flip the tail is made, the data presented thus far do not *prove* that this is the case. For example, in principle there could be other (as yet unidentified) neurons that act in parallel with the LGIs to mediate the tail flip. What further experimental evidence would be required to demonstrate that the LGIs are indeed the only neurons in town that play this critical role? Two types of compelling evidence are described in the paragraphs that follow.

THE LGIs ARE BOTH SUFFICIENT AND NECESSARY. First, the LGIs are both sufficient and necessary for the tail flip response. Two potent lines of evidence support the unique role of the LGIs as subserving a decision-like function: (1) Action potentials of the LGIs are *sufficient* to produce a normal escape response (comparable to one elicited by a tactile stimulus). (2) Action potentials in the LGIs are *necessary* for the normal response to occur.

Concerning the sufficiency of the LGIs, the early experiments of Wiersma showed that direct activation of an LGI with an implanted stimulating electrode gives rise to a tail flip that is within expectable experimental variation, virtually identical to a tap-evoked tail flip. The necessity of the LGIs for the tail flip is more difficult to demonstrate conclusively. However, a clever experiment by Franklin Krasne and his colleague Gene Olson fills the bill. The basic idea of this type of experiment is to *inactivate* the LGIs (preferably reversibly) during a stimulus that would elicit a tail flip. The LGIs could be inactivated by being hyperpolarized with current injected from an intracellular electrode so that they will not fire an action potential in response to the afferent input. If the tail flip is not elicited when the LGIs (only) are inactivated, and in the same experiment the flip is triggered normally when the LGIs are allowed to fire, these results provide strong evidence that the LGIs are necessary for the tail flip. The trick is to find a way to inactivate the LGIs during the behavioral response—no simple matter, since the response is a powerful abdominal flexion that would shatter any microelectrode inserted in the LGIs. The way Olson and Krasne solved this problem was to measure not the behavioral response per se, but rather the motor output from the CNS that would give rise to the tail flip. Specifically, they cut the peripheral nerves going to the fast flexor muscles (so that no behavioral contraction could occur) and recorded extracellularly from the proximal end of the cut nerve so that they could measure the action potentials of the MoG and fast flexor motor neurons coursing through the nerve on their way to the flexor muscles.

The basic arrangement for this thoughtful experiment is shown in Figure 7.11. Sensory input was activated by electrical stimulation of the nerve containing the sensory neuron axons. The response of the LGI within one abdominal segment was recorded intracellularly, while a second electrode in this LGI was used to hyperpolarize it to prevent action potentials from being generated. Another pair of (extracellular) recording electrodes was placed on the nerve cord to make sure that other LGIs from other ganglia were not activated by sensory input during hyperpolarization trials (recall that the LGIs are actually a chain of giant interneurons that are electrically coupled by septal synapses). As mentioned earlier, the presence or absence of an escape response was measured extracellularly from the motor root. The main finding from this type of experiment was that when the LGI was not hyperpolarized and thus allowed to fire in response to the sensory stimulus, a motor response (an "attempted" tail flip) was recorded (Figure 7.11B; note the action potentials in the motor nerve), but when the action potentials of the LGI were blocked with hyperpolarization, there was no motor output (Figure 7.11C). The experimenters

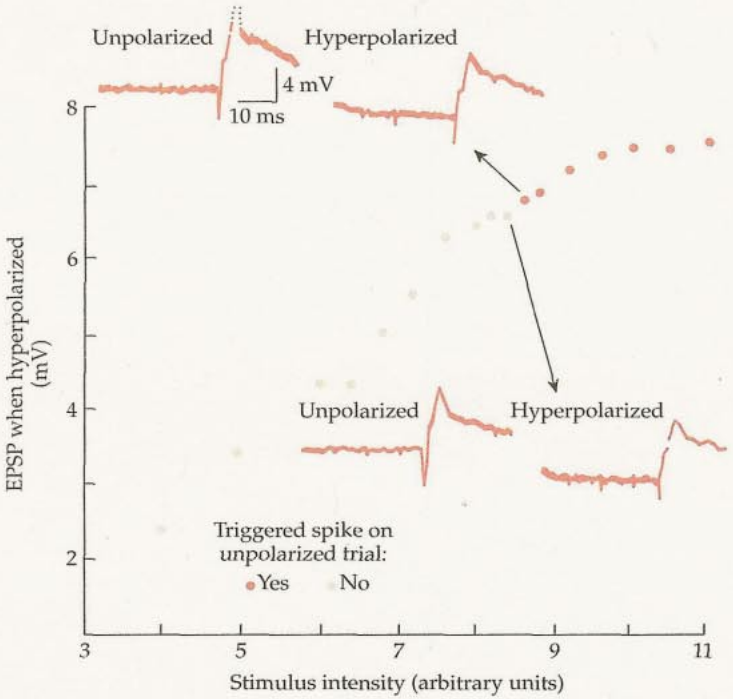


7.11 Testing the necessity of the LGIs for the tail flip
In the experimental setup (A), two intracellular electrodes are placed in the LGI—one for recording the membrane potential (R_{LGI}), the other for passing hyperpolarizing current (Hyp). Two extracellular electrodes are placed on the nerve cord (R_{NC}) and a motor root (R_{Mot}). A stimulating electrode (St) is placed on a nerve root to activate sensory input. (B) When the LGI is not hyperpolarized, the activating stimulus triggers an action potential (AP) in the LGI and output (APs) in the motor nerve. (C) When the LGI is prevented from firing by hyperpolarization, the same sensory input triggers no action potentials in the motor nerves. After Camhi 1984; data from Olson and Krasne 1981.

could repeat this test over and over; whenever the LGI was not allowed to fire, there was no motor output in response to sensory input that normally triggered the response. Thus the LGI, at least under these experimental conditions, can be said to be necessary for the tail flip response.

All the evidence we've discussed here makes the LGI loom large as a candidate for the element in the neural circuit for the tail flip that makes the all-

or-nothing decision to escape. However, another possibility remains. Another all-or-nothing process may be occurring upstream to the LGIs. For example, the sensory interneurons could have a sharp threshold for activation by afferent input: Below this threshold they don't fire, and above it they fire vigorously, thereby activating the LGIs. Olson and Krasne addressed this possibility using the same experimental arrangement as before (Figure 7.11A). They gradually increased the intensity of stimulation of the sensory nerve and examined the net synaptic input of the LGIs. They reasoned that if the LGIs were mere followers of the output of the "real" decision-making units upstream from them, then in a series of sensory stimuli of gradually increasing intensity, the LGI should at some point receive a sudden all-or-none increment in its synaptic input; moreover, this sudden increase should occur just at the threshold for LGI activation and tail flip motor output (see, for example, Figure 7.11B). However, as shown in Figure 7.12, this is not the case. Rather, the input to the LGIs increases quite gradually as the sensory nerve stimulation is gradually increased.



7.12 Change in input to the LGIs in response to increasing stimulation
Input to the LGIs increases in a graded fashion as the sensory stimulation is increased. After Olson and Krasne 1981.

These experiments show that it is indeed the all-or-nothing action potential in the LGIs that gives rise to the all-or-nothing feature of the escape response. This connection explains why there is no such thing as a halfhearted tail flip. The behavior either is not elicited by a sensory stimulus, or a full-blown motor response is triggered. And it appears entirely appropriate to consider the LGIs as decision makers or trigger neurons in generating this striking behavioral response.

LGIs ARE LIKELY CANDIDATES FOR COMMAND NEURONS. The second piece of evidence that LGIs play the decision maker role has to do with the idea of a **command neuron,** a notion that has an interesting history in neurobiology. The term was introduced by a pioneering figure in invertebrate neurobiology mentioned earlier in this chapter—C. A. G. Wiersma—with his colleague K. Ikeda in 1964. They used the term to describe the fact that electrical stimulation of single interneurons in the crayfish evoked coordinated, rhythmic movements of small paddlelike abdominal appendages called swimmerets, which normally exhibit oscillatory rhythmic movements in a variety of behavioral contexts. Following this seminal observation, many other neurons with apparent command function were identified in several species of crustaceans, mollusks, and insects. A common feature of the so-called command cells in all these animals was that activation of these neurons individually, with either intracellular or extracellular electrical stimulation, typically gave rise to some form of complex behavioral output. Thus over a decade or so the notion of command neurons evolved to denote neuronal "push buttons" (as Wiersma called them) that triggered the execution of a coordinated behavioral act.

But the term "command neuron" was used differently by different experimenters, and sometimes there was heated debate about whether a particular neuron deserved entrance into the command neuron "club." Then in 1978 a thoughtful and provocative paper was written by Irving Kupfermann and Klaudiusz Weiss in which they attempted to provide a rigorous definition of a command neuron. They suggested that "the responsibility for a given behavioral response should be attributed to a cell only if its activity is both necessary and sufficient for the initiation of the behavior." They further suggested that the candidate command cell should normally respond to the eliciting stimulus for the behavior, and that activation of the cell (in examining the sufficiency condition) should be done in such a way as to mimic the normal pattern of activation. Kupfermann and Weiss's suggestion for the defining criteria for a command cell sparked lively commentary and debate. At the heart of the debate was the issue of whether any neurons could pass these tough requirements for entry into the command neuron club.

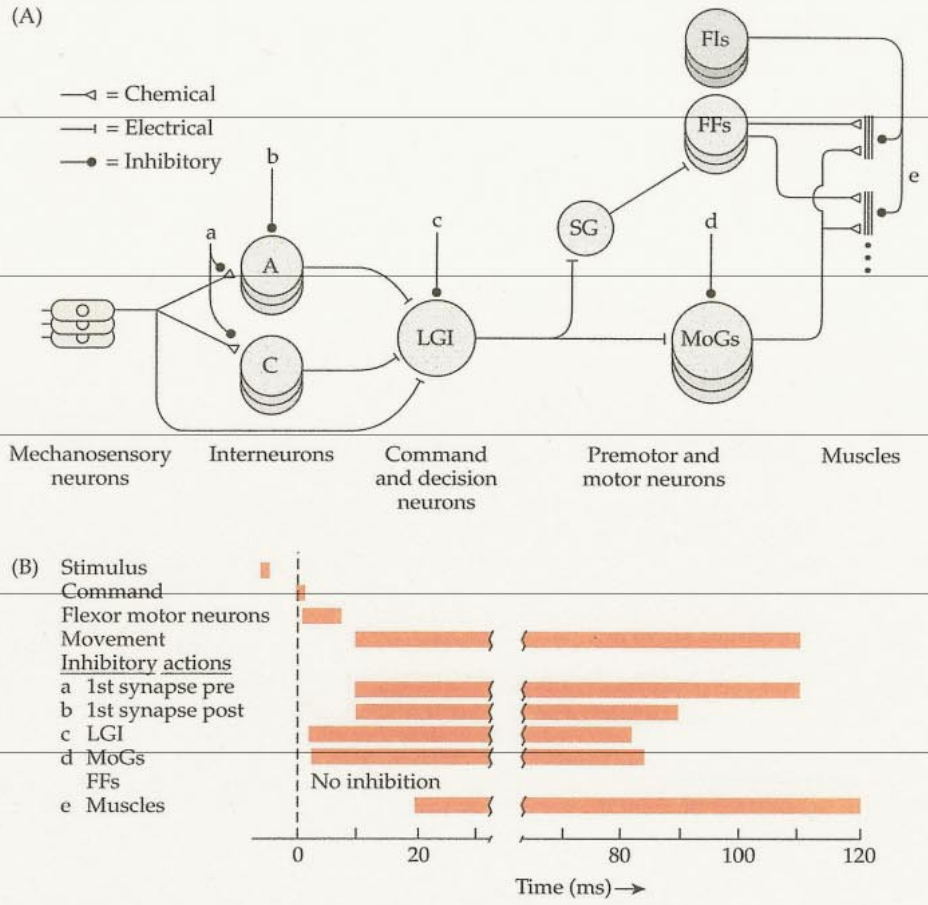
At least a few neurons emerged as leading candidates, among them the LGIs in crayfish. But even in this instance the case was not a foregone conclusion. For example, in the elegant experiments by Olson and Krasne described earlier (see Figures 7.11 and 7.12), the LGIs fulfill both the necessity and the sufficiency criteria, but only under the conditions of their experiment—that is, by

sensory input being activated in the same abdominal segment as the inactivated LGI. Had they activated sensory input to another ganglion, the LGIs in that ganglion would have been activated and a tail flip would have been triggered. Thus no *single* LGI can be called uniquely *necessary* for the behavior. However, since all the LGIs are electrically chained together, they act as a single functional unit. Thus in practice the LGIs clearly subserve a commandlike function. My vote: They're in the club.

COMMAND-DERIVED INHIBITION. Thus far we have considered the rapid flow of excitation through the tail flip circuit as sensory information, funneled through the LGIs, elicits rapid flexion. But, as especially the work of Wine and colleagues has shown, inhibition in this same circuit is of paramount importance in shaping the behavior, in preventing other competing behaviors from being expressed, and in preparing the way for the expression of the next components of the overall escape response. Many of the principal inhibitory actions are initiated by the firing of the LGIs; thus these inhibitory effects are often called **command-derived inhibition.** Command-derived inhibition in the tail flip circuit is *widespread* and differs in its *temporal characteristics*, depending on the site and functional consequences of the inhibitory actions. We will discuss each of these aspects of command-derived inhibition in turn.

The effects of command-derived inhibition are orchestrated to ensure that once the LGIs have fired and generated a flexor motor response, another LGI-mediated flexion will not occur until the first two components of the overall escape response (flexion and reextension, which take about 100 ms) has occurred. The sites where command-derived inhibition is exerted are shown in Figure 7.13A. They include:

1. **The LGIs themselves.** The lateral giant interneurons (as well as the MGIs) are rapidly inhibited following an LGI spike, and this inhibition contributes importantly to the brevity of LGI spike trains (rarely exceeding 3–4 spikes).
2. **Sensory inflow.** Sensory input is inhibited by means of both postsynaptic inhibition of the sensory interneurons and presynaptic inhibition of afferent-to-sensory interneuron synapses. As we will see later, this inhibition is delayed to coincide with the actual flexion movement of the abdomen. Rapid abdominal flexion could easily give rise to water currents, which would reexcite the sensory hairs on the abdomen, potentially triggering another LGI-mediated flip, thereby producing an endless cycle of tail flips. Effectively shutting down the afferent input to the system by command-derived inhibition at this site prevents this potential cycle of responding.
3. **The motor giants.** These major flexor motor neurons are inhibited after sufficient time has elapsed for them to discharge only once. Again, this inhibition prevents another flexor discharge from occurring before reextension can be accomplished. Interestingly, the fast flexor motor



7.13 Location and timing of command-derived inhibition
Command-derived inhibition occurs at several sites and with different delays within the tail flip circuit. (A) The neural circuit for the tail flip, indicating the different sites of command-derived inhibition (a–e). FI = inhibitory flexor motor neuron; see Figure 7.8 for explanation of other abbreviations. (B) Relative time of onset and duration of inhibition at the sites indicated in part A. After Krasne and Wine 1987.

neurons, which would be another logical site for inhibition at this time, are not inhibited.

4. **Fast flexor muscles.** The flexor muscles themselves are inhibited at just about the time when peak behavioral flexion is accomplished, presumably in anticipation of the reextension phase (which, as we shall see, is accomplished in part by inhibition of the antagonistic flexion response).

In addition to inhibition at the sites mentioned here (Figure 7.13A), all of which would contribute to preventing unwanted activation in the flexion circuit prior to reextension, inhibition is rapidly exerted in the extension side of the circuit, to prevent competition with the initial rapid flexion response. Inhibition in the extensor motor system is seen at three sites: (1) the muscle receptor organ (MRO), a stretch receptor that reflexively triggers extension of the abdomen (which, as we shall see, plays a critical role in the reextension response); (2) the fast extensor motor neurons; and (3) the fast extensor muscles.

A final important site of inhibition is in the postural system. In addition to rapid abdominal flexions and extensions used in tail flips, crayfish are capable of slow, tonic flexions and extension involved in postural adjustments; these responses are mediated by separate motor neurons from the fast flexor and extensor systems. The postural system is too slow to participate in escape. Thus both the slow flexor and the slow extensor systems are inhibited during the tail flips. This inhibition is achieved at multiple levels, including (1) inhibition of the slow flexor motor neurons, (2) excitation of inhibitory motor neurons to slow extensor muscles, and (3) inhibition of the sensory receptors and sensory interneurons that provide input to the slow flexor and extensor systems.

As can be appreciated from the discussion here, activity in the LGIs triggers massive, widely distributed inhibition throughout the tail flip circuit. These command-derived inhibitory actions differ from one another in two important ways in terms of their *timing:* (1) Some inhibitory synaptic potentials are delayed in their onset, whereas others are triggered virtually immediately, and (2) some inhibitory synaptic potentials are long in duration, while others are quite short.

In terms of delay, virtually all inhibitory actions generated by the LGIs begin within a few milliseconds of the LGI action potential. However, there are three important exceptions. As can be seen in Figure 7.13B, both presynaptic and postsynaptic inhibition of the first central synapse (the input from the mechanosensory neurons) are delayed by about 10 to 15 ms. In addition, inhibition of the fast flexor muscles is delayed by as much or even a little more. The delay at these three sites is achieved by means of intercalated interneurons between the LGIs and the final site of inhibition.

In considering the overall timing of the onset of inhibition in the tail flip circuit, a clear picture emerges that makes good sense. Early inhibition of the extensor system would clear the way for the flexor activity required to generate the tail flip, whereas delayed inhibition of the flexor muscles themselves would clear the way for the subsequent reextension component of the escape response. The delayed onset of inhibition at the first central synapse would occur in register with generation of the flexion response, thus canceling afferent feedback resulting from the tail flip. In addition to differing in onset time, the inhibitory synaptic potentials generated by LGI activity differ in their duration. Inhibitory postsynaptic potentials (IPSPs) are either of short duration (15 ms or less) or of long duration (20 ms or longer). The role for the location

of short and long IPSPs is straightforward and logical: Short-duration IPSPs occur exclusively in the extension circuit, which makes sense since the extensor elements must be relieved from inhibition and allowed to fire about 20 ms after the flexion command, just in time to begin reextension. In contrast, long-duration IPSPs occur exclusively in the flexion circuit. This also makes sense, since these long IPSPs prevent the recurrence of a tail flip that would interfere with reextension.

At this conclusion of our discussion of command-derived inhibition, it is quite striking to see how massive the inhibitory effects of the LGIs are. They are exerted at every level within the tail flip circuit, from sensory input to the muscles that generate flexion and extension, and by virtue of their onset time and duration, they give rise to a remarkable degree of coordination.

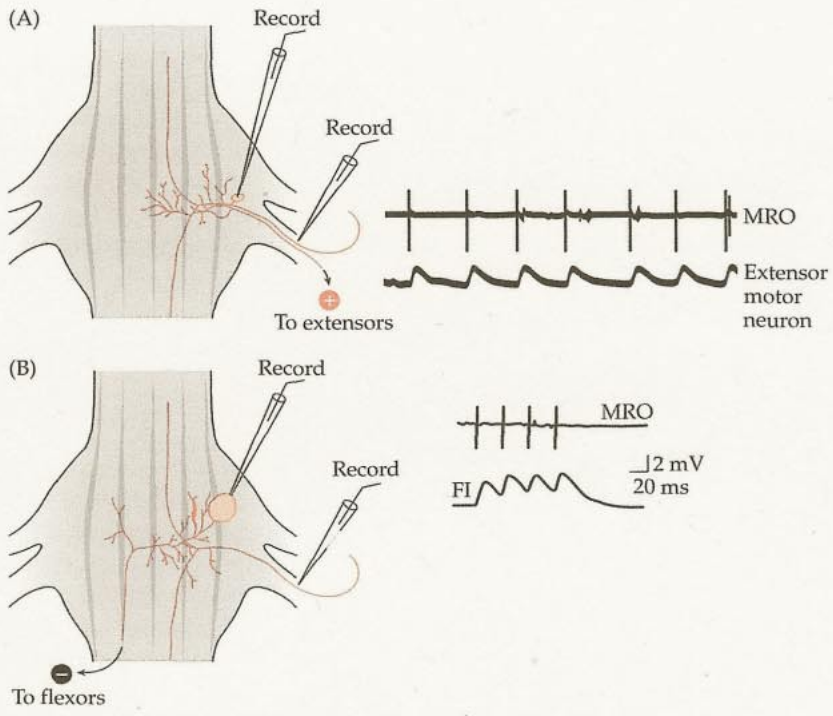
Reextension

Given the importance of the LGIs in initiating the tail flip, it seems logical that the LGIs would also be involved in initiating the second component of the escape response, reextension. But Wine and colleagues have shown that this is not the case. Rather, reextension is due primarily to two sources of excitation to the extensor motor system, each deriving from the sensory input produced during the rapid flexion phase of the escape response.

The first source of sensory input is the **muscle receptor organ** (the **MRO**), which is composed of slender muscles fibers that are located under the dorsal carapace of the abdomen and span the joint between two adjacent abdominal segments. Attached to these muscle fibers are the dendrites of a single sensory neuron. When the abdomen is flexed, the muscle fibers are stretched, causing the sensory neuron to fire. Thus the MRO is a well-characterized stretch receptor; there is one phasic (fast) and one tonic (slow) MRO on each side of an abdominal segment. As we have discussed for other receptors in previous chapters, the MRO is called a proprioceptor because it signals the ongoing state of abdominal stretch to the CNS.

The connectivity of the MRO makes it well suited to mediate an extension reflex. For example, the MRO directly excites fast extensor motor neurons (Figure 7.14A), and it directly excites an inhibitory neuron that inhibits fast flexor muscles (Figure 7.14B). Therefore, the actions of the MRO both initiate extension and inhibit flexion. When these stretch receptors are caused to fire vigorously during the flexion response, they in turn contribute significantly to initiating reextension. Thus the reextension component of the escape response is mediated, at least in part, by a *chain reflex* similar to those described in the locust in Chapter 6.

The second source of sensory input giving rise to reextension is the hair receptors on the abdomen. Physiological experiments show that activation of the hair receptors triggers excitatory synaptic input onto the fast extensor motor neurons. These receptors would be activated as the crayfish flexed its abdomen through the water during the tail flip (exteroceptive input), and their activation would in turn contribute to reextension.



7.14 The muscle receptor organ contributes to the reextension phase
(A) Records from the MRO (extracellular trace, top right) and an extensor motor neuron (intracellular record, bottom right). Each action potential in the MRO is accompanied by an EPSP in the extensor motor neuron. (B) Records from the MRO (extracellular trace, top right) and a motor neuron (FI) that inhibits fast flexor muscles (intracellular record, bottom right). Each action potential in the MRO is accompanied by an EPSP in the FI. After Wine 1984.

Both the MRO and the hair receptors also contribute to the postural system of the crayfish. Thus as mentioned earlier, both of these systems are initially inhibited by LGI activation, to ensure that fast flexion rules the day at the outset of the tail flip. However, once this command-derived inhibition subsides, the excitatory drive producing the reextension response is completely proprioceptive and exteroceptive in nature; the LGIs are passive at this stage of the escape response.

Swimming

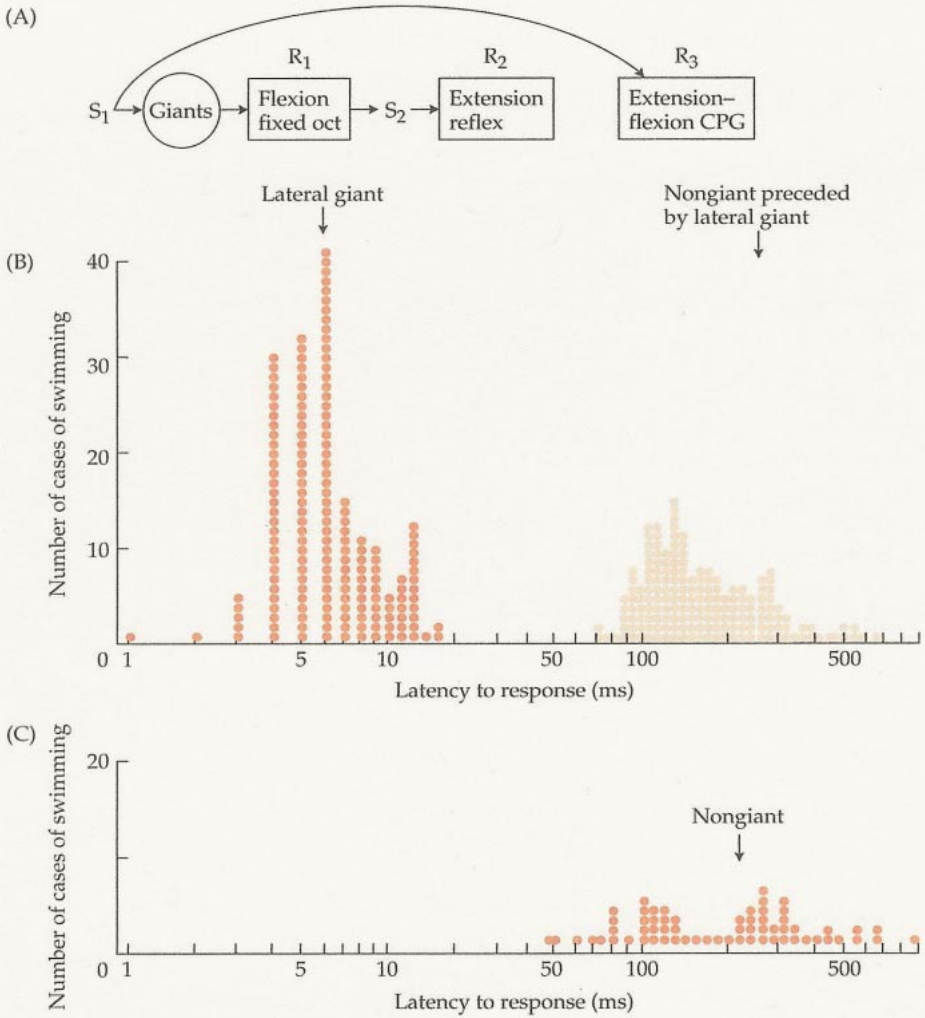
After the rapid flexion and reextension phases of the tail flip are completed, usually a series of nongiant responses, called **swimming**, is observed. These responses invariably consist of an initial abdominal extension, followed by recurrent flexion–extension cycles that propel the crayfish through the water. Perhaps surprisingly, neither LGI-mediated flexion nor reflex-mediated reex-

tension is the trigger for swimming. Rather, swimming is triggered by the same sensory input—activation of the hair receptors on the abdomen—that initiates the tail flip. Thus the LGI and nongiant systems are activated in parallel, but there is a considerable delay (of about 200–300 ms) interposed from stimulus onset until the swimming response. Because swimming does not appear to require sensory feedback for its basic timing or maintenance, it is considered to be mediated by a central pattern generator (CPG).

What is the evidence that the swimming CPG is activated independently from LGI activation and reextension? An elegant series of experiments by Wine and his colleague Heinrich Reichert made the case. They implanted animals with chronic electrodes that could directly activate the giant axons. In these animals they examined the behavioral effects of either directly activating the LGIs electrically, or eliciting an escape response by lightly tapping the abdomen. The critical issue was the incidence of CPG-mediated swimming in these two conditions. The results were clear-cut. Following a tap, which invariably triggered a giant-mediated tail flip, CPG-mediated swimming was observed about 82% of the time. In contrast, after direct activation of the LGIs (which of course always triggered a tail flip), CPG-mediated swimming was almost completely absent, occurring in less than 1% of the cases. These results clearly show that neither activity in the LGIs nor feedback induced from the tail flip is sufficient to trigger the swimming episode. Rather, excitatory input triggered by tactile stimulation is necessary to elicit the CPG-mediated swimming response.

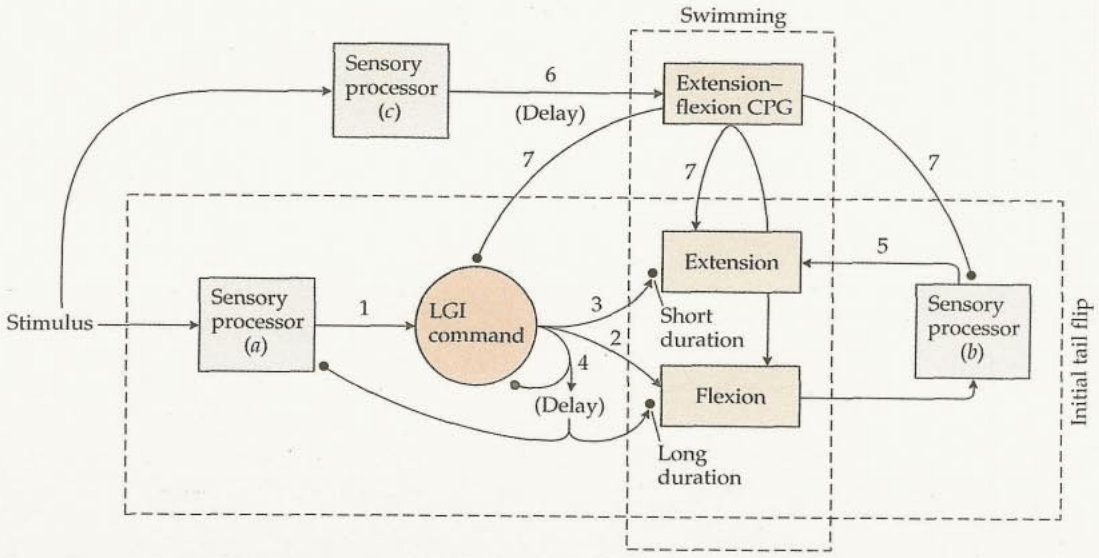
Taken collectively, the results of Reichert and Wine suggest that the relationships between the different units of behavior that make up the escape response can be viewed as shown in Figure 7.15A. A tactile stimulus activates two responses in parallel: the tail flip and swimming, but swimming occurs only after a delay. Reextension is a chain reflex that requires sensory feedback from the tail flip. Further evidence that swimming is independent of LGI activation can be obtained in well-rested crayfish, in which a light tap on the abdomen occasionally triggers a swimming episode in the *absence* of a giant-mediated tail flip. Under these conditions, there is still a considerable delay before expression of the CPG-initiated swimming response (Figure 7.15B and C). Thus the LGI cannot be said to contribute to the long delay before swimming is expressed because the delay is virtually identical (although a bit more variable) when swimming is triggered in the absence of LGI activation. Finally, consistent with the theme that we have seen with command-derived inhibition—the execution of one behavior gives rise to the cancellation of other competing behaviors—during swimming both the LGI system and the reextension reflex are inhibited.

We have now considered in detail the essential features of a full-blown escape response of the crayfish. These features are thoughtfully captured in a comprehensive block diagram devised by Wine, which is shown in Figure 7.16. This flowchart summarizes the major relations among (1) initial flexion, (2) reflex reextension, and (3) the subsequent swimming episode. It is evident from



7.15 Triggering of swimming
Sensory input triggers swimming independently from the tail flip and reextension phases of escape. (A) This schematic diagram shows that a tactile stimulus (S_1) triggers swimming (R_3, with a delay) in parallel with triggering a tail flip (R_1) and a reextension reflex (R_2). S_2 indicates proprioceptive input (arising from R_1) that triggers reextension. (B) The latencies for LGI-mediated tail flips and for nongiant swimming episodes elicited by tactile stimuli are shown. (C) Even when LGI responses do not occur in response to a tactile stimulus, swimming episodes occur with much the same latencies. After Reichert and Wine 1983.

this summary that the initiation of an escape response with an initial latency of less than 10 ms, and the entire tail flip flexion–extension episode that lasts only about 100 ms, takes some fancy footwork in the CNS of the crayfish. Considering how this response is coordinated with a subsequent bout of CPG-



7.16 The complete escape response
This schematic diagram shows the complete escape response (flexion, reextension, and swimming). A tactile stimulus directly activates two sensory processors: One (*a*) triggers the LGI-mediated tail flip (flexion), and the other (*c*) triggers a delayed CPG-mediated swimming response. The second phase (reextension) is triggered by a third sensory processor (*b*), which is activated by input derived from the flexion response. Arrows indicate the flow of information, and numbers aligned with the arrows indicate relative time of occurrence. Solid circles indicate functional inhibition; arrowheads indicate functional excitation. Note that all three phases of escape share common flexion and extension circuitry. After Wine 1984.

derived swimming, it reflects a feat of neuronal engineering of such sophistication as to emphasize the high premium that evolution places on effective escape maneuvers.

Adaptive Modulation of the Escape Response

In our discussion of the escape response thus far, it might appear that, given an adequate sensory stimulus, it is a foregone conclusion that the escape response will be exhibited in short order. But this is not the case. Anyone who has examined this response, from sophisticated researchers in the laboratory to children playing by the side of a stream, quickly comes to appreciate that the response is actually quite fickle. In response to a brisk tap to the tail fan or abdomen, a crayfish may exhibit a tail flip a few times and then suddenly become unresponsive; it may be engaged in other behaviors and appear less concerned about the tail stimulus; or it may on occasion turn and attack the source of the irritating stimulus. Is there any adaptive value to this variability

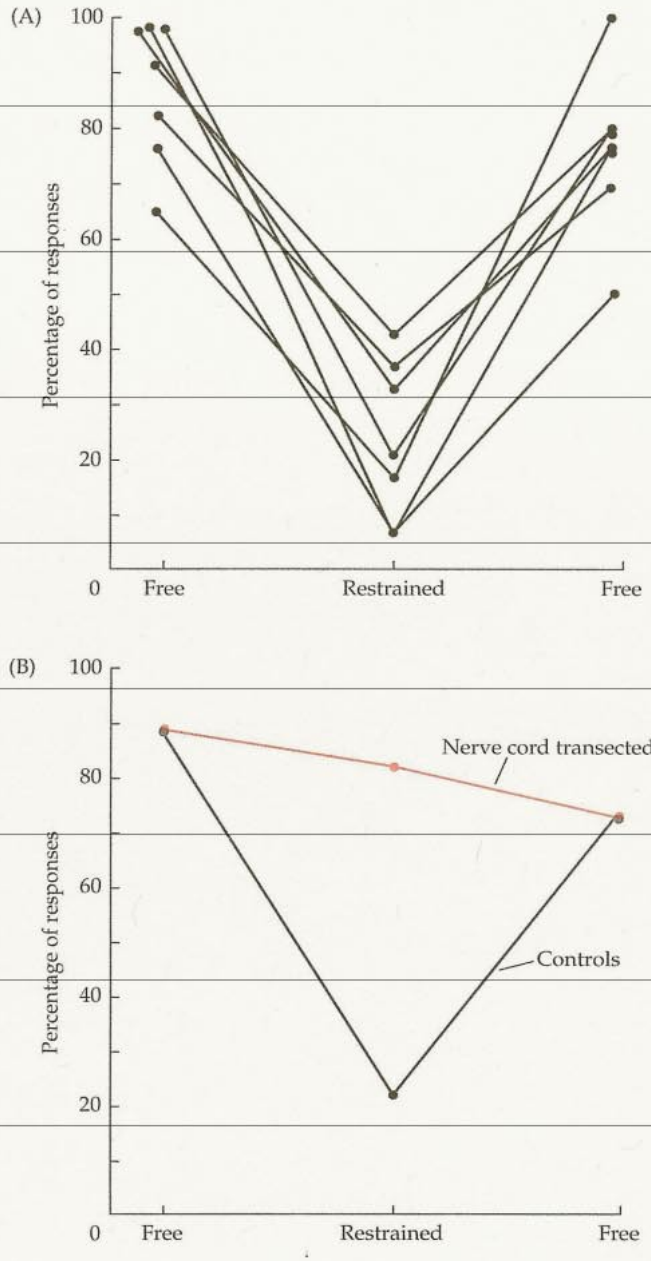
in the escape response? Wine and Krasne put it nicely when they pointed out that "the escape response is too costly to permit its indiscriminate use. It uses a good deal of energy, disrupts many behaviors, and makes the crayfish highly visible to predators with motion-detecting systems." Thus evolution appears to have generated a variety of internal control systems in the crayfish for adjusting the likelihood of escape. In the following sections we will consider three prominent sources of modulation of the escape response: (1) restraint-induced inhibition, (2) motivation, and (3) learning.

Restraint-induced inhibition

One of the most powerful sources of modulation of the LGI-mediated tail flip is restraint of the animal, which dramatically reduces the probability of a response. Researchers who work with crayfish have known for years that holding the animal—for example, by the carapace—greatly reduces the occurrence of evoked tail flips. This observation was formally studied by Krasne and Wine, who measured the threshold of the tail flip reflex when animals were moving freely underwater and when they were held by the thorax in the air. As shown in Figure 7.17A, the likelihood of eliciting an LGI-mediated reflex drops sharply when the animal is being restrained. Krasne and Wine further specified the origin of the inhibition induced by restraint. They found that inhibition was abolished when the nerve cord was severed at the thoracic–abdominal junction (Figure 7.17B). Thus restraint-induced inhibition descends from thoracic and higher levels of the nervous system.

The reflex inhibition described here could be due to a significant *decrease* in facilitation of the LGI-mediated reflex, or conversely a significant *increase* in inhibition from higher levels. These possibilities could be distinguished by examination of the threshold of the reflex following nerve cord transection (which abolishes the behavioral inhibition; see Figure 7.17B). After transection, in both freely moving and restrained animals the threshold for an LGI-mediated tail flip is dramatically *reduced*, indicating that severing the nerve cord removes a potent source of descending inhibition.

In the course of these experiments, Krasne and Wine made several other interesting observations. For example, MGI-mediated reflexes were also suppressed by restraint, and some, but not all, categories of nongiant-mediated escape were reduced. The remaining (noninhibited) nongiant responses could often free the animals from the experimentally imposed restraint, suggesting the possibility that tail flips that could be useful in getting out of a tight squeeze might persist during restraint, while tail flips that would be of no use are suppressed. From this perspective, the control of escape would be shifted away from a short-latency, all-or-none system that is highly stereotyped (such as the giant fiber responses; recall Figure 7.2), to systems such as the nongiant responses that are triggered by a wider range of stimuli and are somewhat more flexible, thereby at least in some circumstances being more likely to yield successful escape. Essentially, Krasne and Wine raised the interesting possibility that restraint causes an adaptive shift from reflexive to more volitional control of escape behavior.



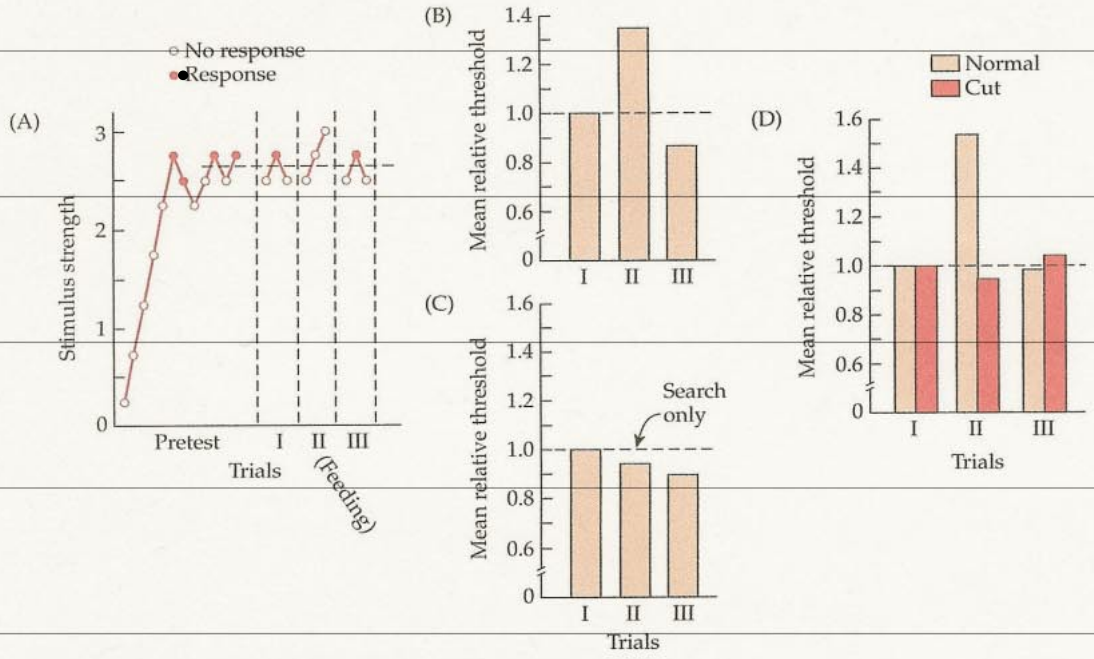
7.17 Restraint inhibits the tail flip response

(A) When an animal is restrained in the air, the likelihood of a tail flip in response to a tactile stimulus is dramatically reduced (middle data points). (B) When the nerve cord is severed at the thoracic–abdominal junction, restraint-induced inhibition is abolished. After Krasne and Wine 1975.

Motivational modulation of escape behavior

Have you ever heard the phrase, "I hate to eat and run, but . . ."? Well, humans aren't the only ones to have this sentiment. Crayfish do too. Specifically, crayfish, like most other animals, often find themselves in a conflicting situation in which they are engaged in one highly motivated behavior, such as feeding,

when another stimulus arises, like a tap to the tail fan, that would normally trigger a tail flip. What to do? Play it safe and escape (but go hungry), or stay the course and eat, with the drawback of not immediately getting out of harm's way? Krasne and his colleague Sunhee Lee have shown that evolution came up with an interesting compromise in the nervous system of the crayfish: Feeding behavior raises the threshold of LGI-mediated escape responses but leaves other escape routes open. In behavioral experiments, Krasne and Lee measured the threshold of LGI-mediated tail flips by gradually increasing the strength of an electrical stimulus to the tail fan (via implanted electrodes). Once threshold had been established, they let the crayfish feed on small pieces of liver for a while, and while the animal was eating they assessed the reflex threshold once again. As Figure 7.18A shows, during feeding the threshold for the response was increased. Interestingly, the suppression of escape appeared



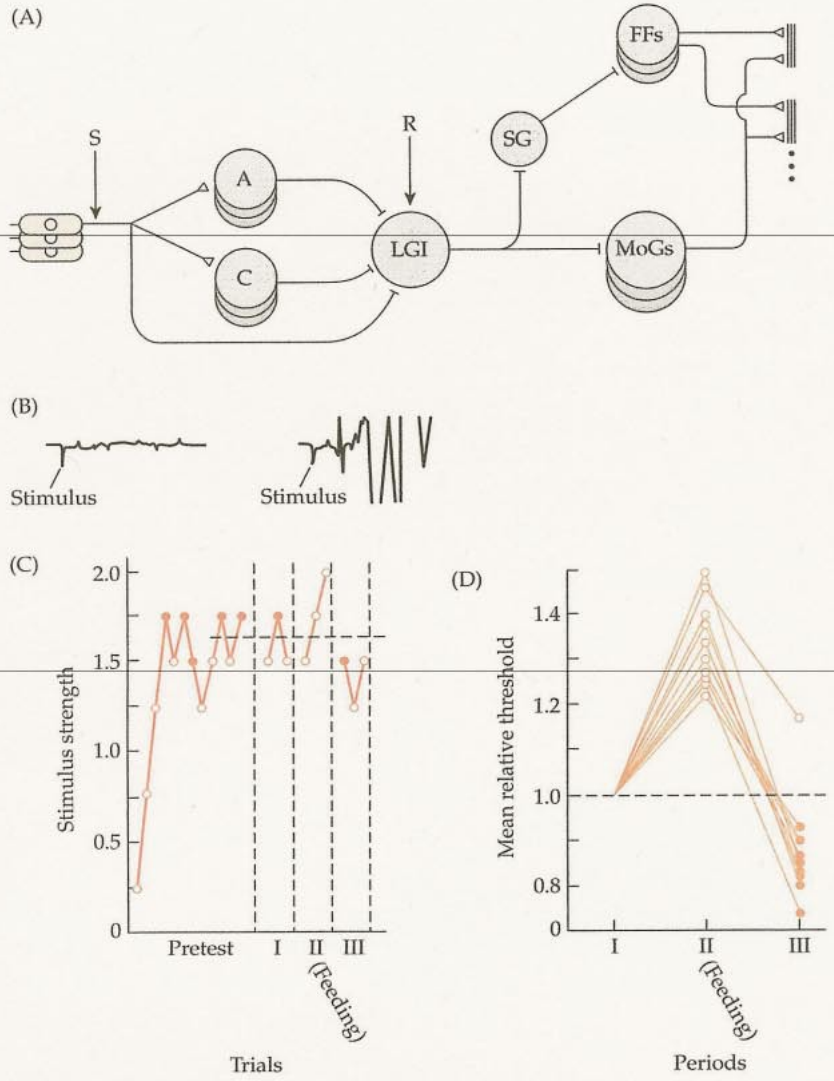
7.18 Feeding inhibits the tail flip response
(A) Gradually increasing the strength of a triggering stimulus finally brings it to threshold for a tail flip (filled circles). While the animal is feeding (trial block II), the threshold for the tail flip further increases (no tail flips occur) from a level (dashed horizontal line) that normally elicits responses in nonfeeding animals (trial blocks I and III). (B, C) The increase in threshold requires that an animal actually be engaged in a feeding response (B), trial block II; simply being in the presence of food (C) does not increase the threshold. (D) When the nerve cord is cut (darker bars) feeding-induced increases in threshold are abolished compared to normal animals (lighter bars). After Krasne and Lee 1988.

related to the actual consummatory behavior in which the animals were engaged because the escape threshold was not affected if animals were actively searching for inaccessible food in the water (compare Figures 7.18B and C). Thus the smell of food or the arousal that it produces is not sufficient to suppress the reflex; the animal must be in the act of eating for the reflex to be reduced. In a final behavioral experiment, it was shown that the suppressive effects induced by feeding originated in thoracic levels or higher, since feeding-induced inhibition of the reflex is abolished in animals whose nerve cords are transected between the abdomen and the thorax (Figure 7.18D). Thus the source of feeding-induced inhibition has features in common with restraint-induced inhibition (see Figure 7.17B).

Where in the tail flip circuit does feeding-induced suppression of feeding occur? Krasne and Lee examined this question by systematically exploring the effects of feeding on different sites in the reflex pathway in animals with chronically implanted electrodes. An example of their results is shown in Figure 7.19, in which they examined transmission from sensory input to the LGI (Figure 7.19A and B). As in the behavioral experiments, they first established the threshold for sensory activation of the LGI (Figure 7.19B); then they explored the effects of feeding on that threshold. They found that during feeding the threshold for LGI activation was significantly elevated (Figure 7.19C and D). In fact, the results examining LGI threshold to sensory input were strikingly similar to the behavioral results (compare Figures 7.18A and 7.19C).

Krasne and Lee went on to examine the effects of feeding on several other sites in the circuit (see Figure 7.19A): sensory input to interneurons A and C, LGI input to the MoGs, and SG input to the fast flexor motor neurons. None were affected. Thus it appears that a single response-dedicated trigger neuron, the LGI, is modulated by feeding. This scheme makes adaptive sense because it leaves intact other escape options, such as nongiant responses, which might make use of some of the same reflex circuitry (e.g., the fast flexor system). Moreover, the suppression of the LGI system is not absolute. It can be overridden if the sensory input is strong enough. Thus evolution has provided a mechanism whereby the crayfish can feed in peace, knowing that it can always recruit effective escape strategies if the going gets really tough.

Before turning to the final section on learning, we can gain an important insight from studies in crayfish about the role of inhibition at different sites within a neural circuit. Throughout this chapter we have seen that inhibition is exerted at many sites in the escape circuit and for many purposes—sometimes to cancel other behaviors (such as preventing extension during flexion), sometimes to raise the threshold of the reflex (such as during restraint or feeding). As we have seen, one predominant site of inhibitory action is the LGIs. In a creative series of experiments, Krasne and his colleague Eric Vu pointed out that two kinds of suppression of LGI responses can be distinguished: (1) One is initiated by the LGIs themselves (command-derived inhibition), which is designed to ensure that a second tail flip cannot be triggered while an ongoing flip is in progress. Since such behavior would be highly mal-



7.19 Feeding increases the threshold for LGI activation
(A) Sites in the tail flip circuit where the stimulus (S) was delivered and where the LGI response (R) was recorded intracellularly. (B) As the stimulus strength is increased, the threshold for action potentials in the LGI is reached (the traces are clipped on the top and bottom). (C) By the same technique as in part B, the threshold for spikes in the LGI (filled circles) is determined. During a feeding episode (trial block II) the threshold is increased such that no spikes are elicited (open circles) at a level (dashed horizontal line) that normally elicits spikes in nonfeeding animals (trial blocks I and III). (D) Average data illustrating the dramatic increase in LGI threshold during a feeding episode. A and B after Krasne and Lee 1988.

adaptive, this form of inhibition should rule the day; it should be *absolute*. In addition, (2) the probability of an escape response is reduced such as we have discussed during restraint or feeding. The process responsible for this suppression—tonic inhibition—is more flexible. For example, as we discussed earlier, although it may be adaptive to continue feeding in the face of a annoying stimulus or modest threat, this inhibition can still be overridden if a tail flip is truly called for; thus this form of inhibition should be *relative*.

Vu and Krasne reasoned on theoretical grounds that an effective way to produce absolute inhibition of the LGIs in the first case and relative inhibition in the second would be to place the inhibitory synapses in different locations on the LGI. Specifically, absolute inhibition could be best achieved by inhibitory synaptic input being placed "proximally," near the spike-initiating zone (where it would be in a perfect place to "cancel" any output from the LGI), while relative inhibition could be achieved by the placement of inhibitory synapses "distally" on LGI dendrites, where the inhibition would have to compete with other inputs for "control" over LGI firing. Remarkably, these predictions were found to be true: Under conditions of command-derived inhibition, the inhibitory synaptic input to the LGIs was exerted at proximal synapses, whereas restraint-induced tonic inhibition was produced at distal synapses. This imaginative study highlights the point that inhibitory effects can have different functional consequences not only when they are exerted at different sites in a neural circuit, but also when they are exerted at different sites on a single neuron!

Modulation of escape behavior by learning

One of the most important things virtually all animals must learn is not only what is important in their world, but also what is not important. Thus we typically do not feel the clothes on our bodies, we readily "get used to" the constant hum of a refrigerator in our kitchen, and we can even learn to ignore loud repetitious noises such as road repair work outside our windows (even loud noises that initially would alert or startle us). These are all instances of a simple form of learning called **habituation,** a process described as the reduction in a behavioral response produced by repeated stimulation. This simple form of learning is observed in virtually all animals—and the crayfish is no exception. For example, if the LGI-mediated response is repeatedly elicited—for example, by taps to the abdomen every minute or so—the probability of a response progressively declines. The learning lasts several hours and is even detectable the next day. Thus crayfish, like every other animal, display the clear ability to habituate.

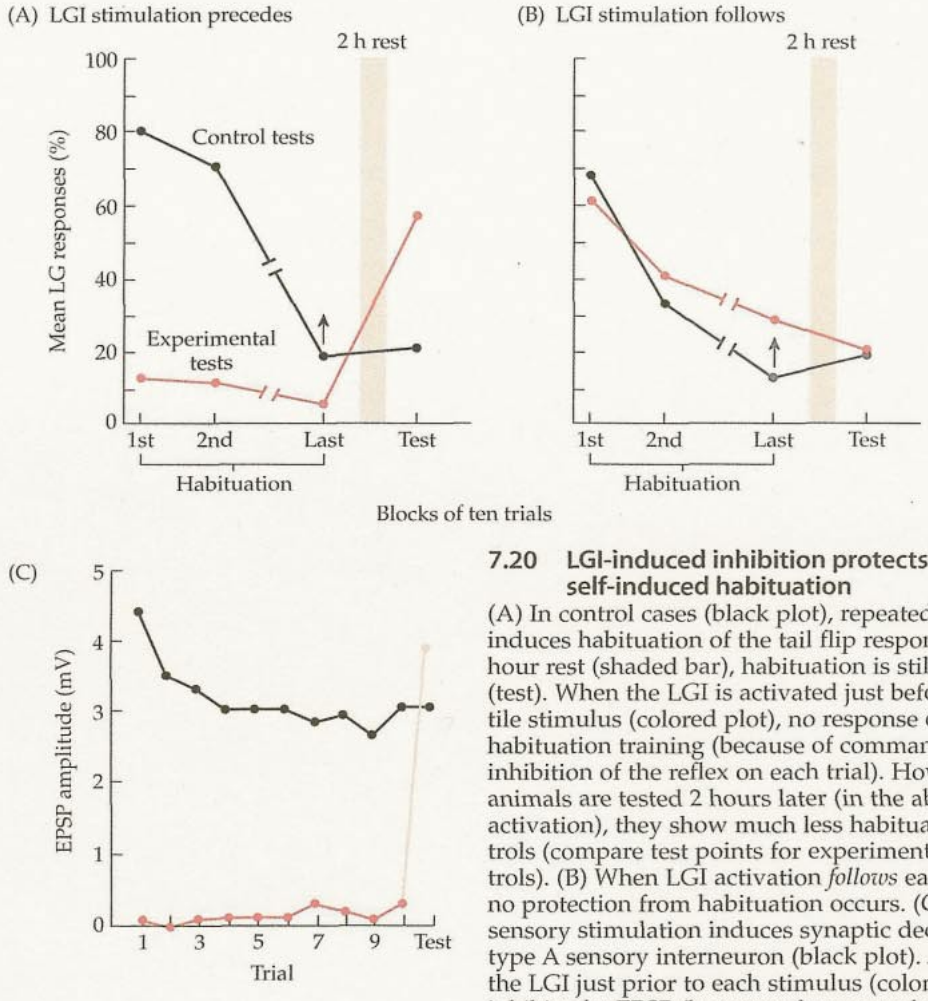
But now we have a problem. Say a crayfish is tapped on the tail fan and responds by flipping its tail. During that escape response, the sensory hairs on its abdomen are going to be vigorously activated by the surge of water across them during the flip. This sensory input is thus going to be repeated and prolonged—just right to produce habituation of the response. So why doesn't a single tail flip give rise to self-induced habituation? If it did, it could be costly

because as soon as the crayfish landed from its initial escape, if it had habituated itself it would be potentially vulnerable to another threatening stimulus that the crayfish might not want to ignore. So the problem is, how can the crayfish distinguish between repeated input arising from an outside stimulus and repeated input due to its own escape response? Once again, evolution has come up with a highly creative solution. Self-habituation is prevented, at least in part, by command-derived inhibition.

That the occurrence of an LGI-mediated reflex can protect against habituation was demonstrated by examination of habituation under two conditions: In one condition animals received repeated tap stimulation as usual for habituation studies. In the other condition, the same animals received identical repeated taps, but just before each tap their LGIs were activated (with implanted electrodes). In either condition, after comparable numbers of habituation trials, 2 hours later the animals were tested to assess the amount of habituation they exhibited. The results are shown in Figure 7.20A.

Under control conditions, animals showed normal habituation (to about 20% of the normal response). Also as expected, under the condition of LGI preactivation, animals showed practically no response to the repeated taps, since each tap was preceded by giant fiber activation, which produced command-derived inhibition of the tap-evoked response. The critical observation was during the 2-hour test. Under control conditions, habituation was still unchanged (responses still were down to about 20%). Under conditions of LGI preactivation, however, responses were significantly less habituated (animals showed responses on about 60% of trials). In an important control experiment, there was no difference between tests following taps alone and those following taps that were then *followed* by activation of the LGIs (Figure 7.20B). These behavioral results clearly reveal an elegant solution to the problem of self-induced habituation: Whenever a tail flip is initiated by an LGI, concomitantly the mechanisms underlying habituation are suppressed.

A final important question is, What are the mechanisms of habituation of the tail flip? One candidate mechanism that has been long appreciated by workers studying crayfish is the depression of synaptic transmission at the first central synapse onto the sensory interneurons (see Figures 7.6 and 7.8). For example, extensive research by Robert Zucker has shown that repeated sensory input, evoked either by tapping of the abdomen or by electrical stimulation of the sensory nerve, gives rise to progressive synaptic depression in A-type and C-type sensory interneurons. Thus as the model would have it, repeated taps produce habituation because the LGIs are less and less likely to fire because of the progressively reduced excitatory input onto them. Furthermore, just as protection from habituation can be produced by preceding LGI activity (see Figure 7.20A), so too does LGI firing just prior to sensory activation protect from synaptic decrement (see Figure 7.20C). The mechanism of this protection at a synaptic level appears to by command-derived presynaptic inhibition at this synapse (site a in Figure 7.13). More recently, another mechanism of habituation of the tail flip has been identified by Krasne and his colleagues. They examined cellular changes in freely moving intact crayfish and, under these



7.20 LGI-induced inhibition protects against self-induced habituation

(A) In control cases (black plot), repeated stimulation induces habituation of the tail flip response. After a 2-hour rest (shaded bar), habituation is still evident (test). When the LGI is activated just before each tactile stimulus (colored plot), no response occurs during habituation training (because of command-derived inhibition of the reflex on each trial). However, when animals are tested 2 hours later (in the absence of LGI activation), they show much less habituation than controls (compare test points for experimentals and controls). (B) When LGI activation *follows* each stimulus, no protection from habituation occurs. (C) Repeated sensory stimulation induces synaptic decrement in a type A sensory interneuron (black plot). Activation of the LGI just prior to each stimulus (colored plot) inhibits the EPSP (because of command-derived presynaptic inhibition; see site a in Figure 7.13A and B). In the test period (in the absence of LGI activation), however, the EPSP exhibits less decrement than the control EPSPs. Thus LGI activation prior to each sensory stimulus protected against synaptic decrement (which is presumed to contribute to habituation of the tail flip response). A and B after Wine, Krasne, and Chen 1975; C after Bryan and Krasne 1977.

conditions, found that a buildup of tonic, descending inhibition in the circuit, evoked by repeated activation of the tail flip, appeared to be the predominant mechanism of habituation. Thus in the crayfish as in other animals, a family of cellular mechanisms is likely to contribute to even a very simple form of learning such as habituation.

In conclusion, in modern neuroethology few examples rival the crayfish as a system in which the neural circuitry underlying a complex behavior is understood both in terms of its functional anatomy and in terms of its principles of operation. This system is in one sense simple, making use of giant neurons to initiate a highly reliable and quite stereotyped behavioral response. But upon closer inspection it is a highly sophisticated system in which a rapid neuronal command decision gives rise to massive coordination of both complementary and competing neural machinery, a decision that is mediated by a single impulse lasting a fraction of a second in a single cell but that unleashes a coordinated behavioral response lasting orders of magnitudes longer than the triggering event. Little wonder that the crayfish is such an accomplished escape artist.

Summary

In response to an abrupt tactile stimulus, crayfish exhibit a striking *tail flip* escape response. The form of the response differs depending on the site of stimulation. A stimulus to the tail fan or abdomen gives rise to a forward tail flip resembling the first half of a somersault. A stimulus to the front of the animal produces a tail flip that propels the animal directly backward. Each of these responses is triggered by a pair of giant axons that travel the length of the central nervous system. The first response is mediated by the *lateral giant interneurons* (*LGIs*), the second by the *medial giant interneurons* (*MGIs*). Both responses are achieved with a very short latency, beginning within a few milliseconds of the initiating stimulus, and both responses involve massive flexion of the abdomen. A third form of escape response, which usually follows a giant-mediated tail flip, is slower in onset and more variable in form. It usually takes the form of alternating extension and flexion, which produces swimming.

The best-understood response is that mediated by the LGIs. These neurons receive sensory input from sensory hairs located on the abdomen and in turn produce widespread activation of the flexor system. Experiments show that the LGIs are both necessary for the initiation of a tail flip and sufficient to produce the response when they are directly activated electrically. Thus they are considered *command neurons*, or trigger neurons, that act as decision units to trigger an escape response.

In addition to producing rapid excitation of the flexor system, the LGIs give rise to *command-derived inhibition*, which is extremely widespread in the tail flip circuit and serves to cancel competing responses (such as extension) and to clear the way for the subsequent expression of other components of the escape response, such as reextension and swimming. Reextension is produced by a chain reflex; that is, it is triggered by sensory feedback (reafference) arising from the generation of the initial flexion component of the tail flip. The sensory input comes from two principal sources: stretch receptors on the abdomen (the muscle receptor organs, or MROs) and sensory hairs on the tail fan and abdomen. Swimming is produced by a *central pattern generator* that is activated by senso-

ry input in parallel with the tail flip, but with a prolonged delay so that it is expressed at the completion of the flexion and reextension components.

The tail flip response is modulated by a variety of influences. For example, restraining the animal by the carapace gives rise to tonic inhibition of the tail flip. Likewise, when the animal is engaged in feeding, the tail flip is suppressed. In both cases, inhibition originates at higher levels of the nervous system (above the abdominal segments) and acts rather selectively to inhibit giant-mediated responses, leaving at least some types of nongiant responses unaffected. Finally, simple forms of learning modulate the response. For example, in response to repeated activation, the probability of an escape response declines; that is, the response habituates. To prevent self-induced habituation caused by repeated sensory input during a tail flip, evolution has come up with a creative solution: Whenever a tail flip is produced by LGI activation, the mechanisms underlying habituation are concomitantly suppressed. In this way, protection from habituation is generated at the same time the escape response is triggered.

Recommended Reading

Krasne, F. B., and Wine, J. J. 1987. Evasion responses of the crayfish. In *Aims and Methods in Neuroethology*, D. M. Guthrie (ed.), pp. 10–45. University of Manchester Press, Manchester, England.

Reichert, H., and Wine, J. J. 1983. Coordination of lateral giant and non-giant systems in crayfish escape behaviour. *Journal of Comparative Physiology. A, Sensory, Neural, and Behavioral Physiology* 153: 3–15.

Roberts, A., Krasne, F. B., Hagiwara, G., Wine, J. J., and Kramer, A. P. 1982. Segmental giant: Evidence for a driver neuron interposed between command and motor neurons in the crayfish escape system. *Journal of Neurophysiology* 47: 761–781.

Wine, J. J. 1984. The structural basis of an innate behavioural pattern. *Journal of Experimental Biology* 112: 283–319.

Wine, J. J., and Krasne, F. B. 1982. The cellular organization of crayfish escape behavior. In *The Biology of Crustacea*, D. E. Bliss, H. Atwood, and D. Sandeman (eds.), Vol. 4: *Neural Integration*, H. Atwood and D. Sandeman (eds.), pp. 242–292. Academic Press, New York.