Crayfish Swimming: Alternating Motor Output and Giant Fiber Activity

Abstract. *Many workers have suggested that the crayfish giant fibers trigger swimming movements or tail flips during escape responses. Recordings from intact animals show that this is often not the case; both swimming and single tail flips can occur in the absence of giant fiber activity. Swimming movements and tail flips are coordinated by neural mechanisms not involving the giant fibers. When giant fibers are active, they may trigger the first flexion in a swimming sequence, initiate a single tail flip, or synchronize the muscular activity in the several segments of the abdomen, but they are not a necessary part of the neural oscillator which drives swimming.*

Swimming and tail flipping are not the most frequently used forms of locomotion in crayfish, but they are used for escape when the animal is startled or highly excited. A tail flip is a single strong flexion of the abdomen, and a swim is a series of flexions at frequencies of 5 to 15 per second. Both escape modes can rapidly propel the animal in a general backward direction. The complex, spiral muscles responsible for fast flexion (*1*) fill most of the abdomen (*2*). Stimulation of central giant fibers elicits action potentials in the largest flexor motor neuron and some of the smaller neurons that produce twitches in fast flexor muscles (*3, 4–8, 14*). Two or more cord giant fibers connect to every fast flexor motor neuron in the third abdominal ganglion (*8*).

There is already some evidence indicating that giant fiber activity may not be absolutely essential for tail flipping and swimming in crayfish. Some of the smaller motor neurons are excited by cord inputs other than giant fibers (*5, 6*); in dissected crayfish strong sensory stimulation often elicits single abdominal flexions without the participation of giant fibers (*9*); crayfish which presumably lack input to medial giant fibers because circumesophageal connectives have been severed can still swim (*10*). However, when it is considered that giant fibers in many invertebrates are associated with startle or escape responses (*7, 11, 12*), a reasonable functional interpretation for crayfish is that the cord giant fibers are intimately involved in swimming and tail flipping in the intact animal, and this idea has often appeared in the literature on crayfish neurophysiology (*3, 6–8, 11, 13*). The converse idea that they are not needed for swimming is virtually unrecognized.

This study was undertaken in order to determine the extent to which crayfish cord giant fibers mediate swimming or tail flipping in freely behaving animals, and in order to describe the motor output during swimming. The latter has never been characterized. Analysis of the motor output gives circumstantial evidence about giant fiber involvement, and furthermore, electromyograms provide a convenient monitor of the swimming movements. Without some measure of the behavior itself, records from the nerve cord cannot be interpreted. Therefore, evidence from electromyography will be presented first.

Crayfish can begin to swim with the abdomen either flexed or extended. In the former case they must extend the abdomen before the first tail flip. This extension can sometimes be visually observed as a gradual preparation for the first tail flip. During swimming the tail extends rapidly between flexions. It has not been shown previously whether the preparatory or repeating extensions are produced by fast activity of extensor muscles, or whether they are due merely to combinations of tonic postural motor output and elastic rebound.

In order to characterize the motor output during swimming, I recorded muscle action potentials with 50- or 100-μm wires, insulated except at their tips, inserted into fast flexor and extensor muscles of abdominal or last thoracic segments. Specimens of *Procambarus clarkii* and another species of the same genus were used. Experiments were done with small animals (6 to 10 cm from the tip of the rostrum to the end of the telson), because they swim more readily than large ones. Positions of the electrodes were determined by dissection after each experiment. Swimming and tail flipping were usually elicited by anterior tactile stimulation.

Fast extensor motor activity does alternate with fast flexor discharge, and swimming sequences may begin with either fast flexions or fast extensions (Fig. 1). When electromyographic records are coordinated with visual observations, it can be seen that swimming starts with an extension when the abdomen is tonically flexed, and with flexion when the abdomen is extended. Giant fibers probably do not excite fast extensor motor neutrons (*14*). There-

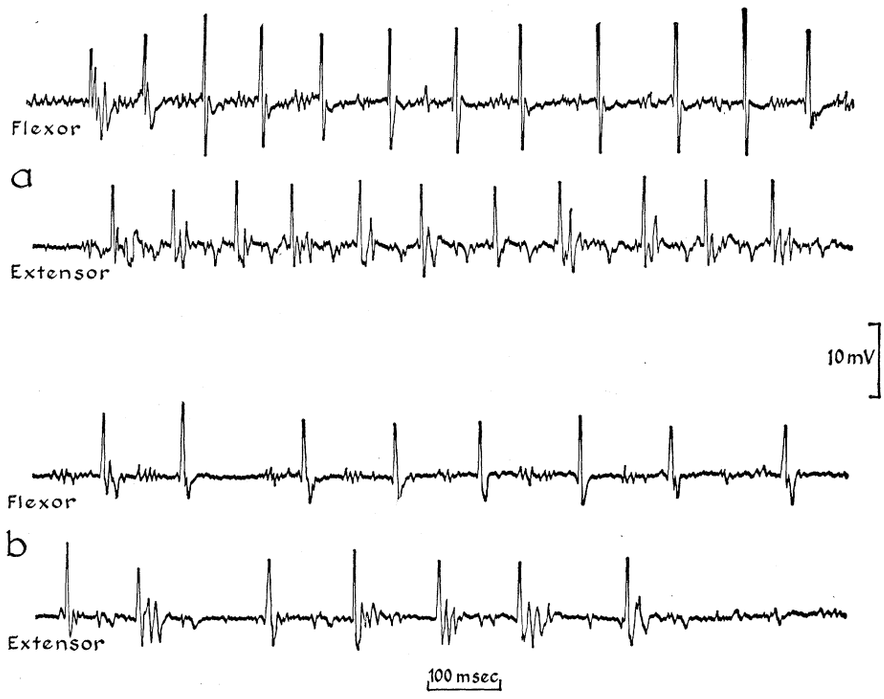

Fig. 1. Fast flexor (trace 1) and fast extensor (trace 2) myograms during swimming. (a) Swim began with flexion. (b) Swim began with extension. A small amount of cross-talk from flexors is visible in extensor myograms. Flexor and extensor myograms are from the third and first abdominal segments, respectively.

fore, that either swimming or single tail flips may begin with a fast extension implies that giant fibers do not always initiate either swimming or tail flipping.

In both flexor and extensor muscles the action potentials reveal a degree of variability of motor output pattern. Either may exhibit a burst of several small or large potentials (or both) in any order in each swimming cycle (Fig. 1). In any one abdominal segment, the extensor motor activity is out of phase with respect to the flexor cycle by an average of about 0.6 of that cycle, but this phase varies widely. Extensor activity can be at a very low level, and occasionally extensor muscle action potentials are undiscernible in the myograms (Fig. 1b). Activity of homologous muscles in different segments is approximately, but not exactly, synchronous. Flexors contract more exactly synchronously than extensors do. The total range of latencies between times of firing of the most distant abdominal flexors in these small animals was about 5 msec, whereas extensor time differences between distant segments were up to several tens of milliseconds. During strong swimming, extensor muscles tend to fire earlier in the more anterior abdominal segments. This tendency should give rise to an unfolding of the abdomen which minimizes water resistance during the extension part of the cycle. But flexor, and especially extensor, intersegmental latency is highly variable with respect to the mean value.

The amplitude of the largest flexor potential varied from cycle to cycle. It sometimes remained approximately constant, but often either grew or diminished by a factor of over 5 in subsequent cycles of the same swim. If the giant fibers triggered the major flexor motor neuron discharge, the first swimming cycle would be expected to have a near maximum flexor action potential. The synapse between giant fibers and the largest flexor motor neuron is of the relay type (*4*), and an impulse in this flexor motor neuron produces a large muscle action potential with the first impulse in a train, but antifacilitation occurs on subsequent impulses (*15*). The variability in the size of the amplitude of the flexor muscle potential from cycle to cycle suggests that giant fibers are not active in every cycle of flexion.

That giant fibers do not necessarily control swimming was verified by recording nerve cord potentials, via a flexible suction electrode attached to the nerve cord while the animal swam (Fig. 2). The two medial and the two segmentally septate lateral giant fibers extend from the brain to the most caudal abdominal ganglion. Control stimulations were done in order to determine the amplitude of the potentials produced by the giant fibers; wires were inserted into the area of the circumesophageal connectives (Fig. 3c), or suction electrodes were applied directly over giant fibers in each connective. Giant fiber spikes were usually much larger than other cord potentials. The electrotonically connected lateral giant fibers generally fire synchronously and produce a larger potential than do the medial giant fibers.

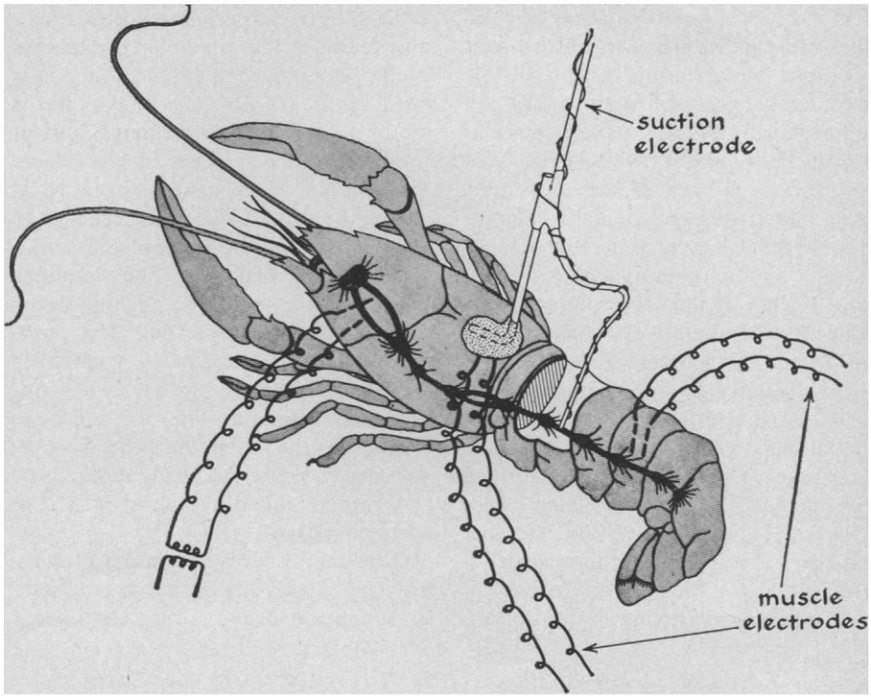

Fig. 2. Method used to record nerve cord and muscle potentials in a swimming crayfish. Tissues dorsal to the cord in abdominal segments 2 and 3 and were removed, and a flexible suction electrode mounted on the dorsal thoracic exoskeleton was applied to the cord on its dorsal surface just over the giant fibers.

In general, the giant fibers were not active during either single tail flips or swims. When giant fiber impulses did occur, they could usually be shown to be from the lateral giant fibers. Sometimes giant fiber impulses accompanied the first abdominal flexion in a swimming sequence, but often during low frequency swimming they occurred only in later cycles (Fig. 3, a and b). Occasionally the lateral giant fibers produced two closely spaced impulses in a single flexion half-cycle. The giant fibers seemed to have a very high behavioral threshold, and the relatively low rate of occurrence of giant fiber impulses in these experiments could be due in part to an habituated or weakened state of the animal after surgery. However, the results do demonstrate that crayfish can swim and flip without the participation of cord giant fibers.

This fact, together with the observation that the occasional presence of giant fiber impulses was not obviously correlated with increases in the amplitude of the flexor muscle action potentials, raises the question of what function giant fibers do have in crayfish swimming. Two kinds of experiments were done in an attempt to determine possible contributions of giant fiber potentials to the neural machanism that generates swimming.

In the first kind of experiment, simultaneous measurements were made from the nerve cord and from flexor muscles in anterior and posterior abdominal segments (Fig. 2). When lateral giant fiber potentials were not involved in swimming or flipping, the anterior abdominal flexor potentials preceded or succeeded the posterior potentials by up to 5 msec. But when lateral giant fiber impulses did occur, the anterior potentials preceded the posterior potentials, most often by only 2 msec. This evidence suggests that the lateral giant fibers may function during slow swimming to synchronize the flexor motor neuron activity more exactly.

In the second kind of experiment, stimulating electrodes were placed near the giant fibers in the region of the circumesophageal connectives (Fig. 2), and artificially evoked lateral and medial giant fiber impulses were interpolated into various phases of the swimming cycle. Stimulation of excitatory inputs to a rhythmic neural generator can affect the amplitude or phase of its output. When impulses were interpolated up to 20 msec before an expected flexion, flexion often occurred immediately. But when extra giant fiber impulses occurred during other parts of the cycle, they usually did not cause immediate flexor muscle action potentials, nor did they have large effects on later cycles of activity. These results indicate that cord giant fibers provide only weak input, if any, to the neural mechanism that generates swimming motor output.

This latter experiment also reveals one facet of the rhythmic mechanism itself. Interpolated giant fiber impulses most often did not give rise to flexor motor output at all, even though in quiet preparations the synapses of giant fibers to the largest motor neuron appear to be of the 1 : 1 relay type. Recordings of cord activity during interpolated stimulations verify that giant fiber impulses did indeed occur without eliciting flexor muscle potentials (Fig. 3c). The probability that an interpolated giant fiber impulse will elicit a flexion is low immediately after one flip, and increases throughout the swimming cycle. This result suggests that the flexor motor neurons are inhibited during part of the swimming cycle.

That the motor neurons and muscles are not simply in a refractory state is demonstrated by another control experiment. The giant fibers were stimulated at swimming frequency and extra stimulations were interpolated. Large flexor muscle potentials were in this case associated with every giant fiber stimulation. Only when the natural swimming oscillator is running are the large flexor motor neurons incapable of responding to interpolated giant fiber impulses.

Taken together, all of the results indicate that the giant fibers are not necessary for swimming. There must be a neural oscillator which drives alternately the sets of antagonistic muscles, but the giant fibers are neither a necessary part of that oscillator, nor can they reset the swimming cycle markedly. One of their functions in swimming may be only to further synchronize the flexor motor neuron activity. It appears that the giant fibers do play a natural role in triggering swimming (*16*), but they are not needed even for this. The swimming oscillator mechanism includes inhibition of the flexor motor neurons during part of the cycle.

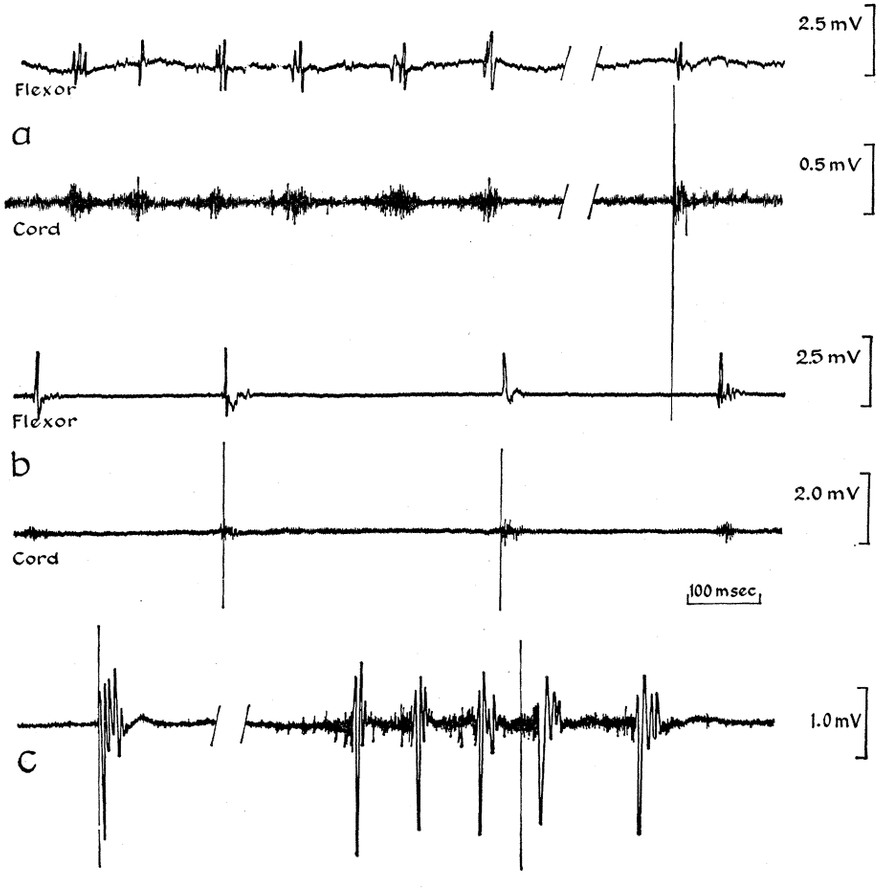

Fig. 3. Flexor muscle activity and nerve cord potentials during swimming. (a) A swim in which no giant fiber potentials occurred, and a single tail flip mediated by a medial giant fiber impulse in the same crayfish. (b) A swim in which lateral giant fiber impulses occurred in two out of four flips. (c) A single tail flip in which stimulation of the lateral giant fibers (interpolated into the third swimming cycle) did not elicit discernible flexor muscle potential. The implanted wire recording electrode was hooked around the nerve cord and was only partially insulated from flexor muscle potentials by Vaseline. Extra giant fiber impulses were stimulated via wires inserted into the circumesophageal region.

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