subjects (Fig. 1). These results agree with our previous finding that the pyrophosphate content of fibroblasts cultured from skin and fibrocartilage of a chondrocalcinosis patient was twice that of fibroblasts from two gouty patients and two normal volunteers (*12*). The fibroblasts of one family member with no overt symptoms had a pyrophosphate content similar to that of fibroblasts from the experimental subjects, and detailed reexamination revealed clinical signs in the knee and radiological evidence of calcified cartilage in the knees, pubic symphysis, and coxofemoral joints. Thus the high value indicated the presence of the gene for chondrocalcinosis before its clinical expression was noted.

This demonstration of a deranged pyrophosphate metabolism in a large kindred is in keeping with the genetic nature of chondrocalcinosis and is correlated with the demonstration by x-ray diffraction of calcium pyrophosphate in triclinic crystalline form in two affected family members (*15*). The presence of a high pyrophosphate content in fibroblasts may explain the calcification found in tendons, joint capsules, and fibrocartilage (*16*). The severity of the disease in this family, in which only one of the parents was affected, raises questions about the clinical features that might be found in families in which both parents are affected. Finally, the finding of this metabolic abnormality in lymphoblasts suggests that lymphoblast cell lines, particularly those cultured from the homozygote, might be used to further study the metabolic derangement underlying chondrocalcinosis.

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Different Command Neurons Select Different Outputs from a Shared Premotor Interneuron of Crayfish Tail-Flip Circuitry

Abstract. *In the crayfish a bilateral pair of interneurons (the I3's) are involved in the generation of two types of tail-flip escape responses, one mediated by giant neurons and the other by nongiant circuitry. The I3's make a variety of output connections with the motoneurons and with other interneurons involved in tail flipping. The motoneuronal outputs include strong synapses on telson flexor motoneurons, whose activity during tail flips mediated by lateral giant fibers would be maladaptive. The lateral giants always drive the I3's, but also drive inhibitory neurons that prevent the undesirable outputs of the I3's while permitting their adaptive outputs to be expressed. It is often adaptive for tail flips initiated by nongiant circuitry to utilize the telson flexor muscles that I3 strongly excites. During such tail flips I3 is often fired, and this firing is important in driving the telson flexors.*

Great economies in the amount of nerve circuitry needed to generate behavior can be achieved by systems of hierarchical control in which complex movements are synthesized from a basic set of movements of rather general utility. Still greater economy could be attained if these basic motor patterns, rather than being fixed and merely callable by higher level controllers, were altered somewhat by those controllers to produce a range of movements. Thus one flexible movement-producing subroutine could play the role of many fixed ones (*1*). However, evidence for alteration of subroutines—in particular, evidence for alterations in their "logical structure" (alterations in what effectively drives or suppresses what)—has mostly been indirect (*2*).

Here we provide direct evidence for such alteration by reporting the discovery of a pair of crayfish motor interneurons that are used in the production of several types of tail-flip escape responses and whose pattern of effective outputs is adaptively altered according to the type of tail flip being produced. This bilateral pair of neurons, the I3's, was discovered as an apparent participant in the production of tail flips mediated by the crayfish's medial giant and lateral giant command neurons (*3*). These escape reactions are rapid, stereotyped responses to sudden stimulation. The medial giants respond to rostrally located stimuli and command a pattern of abdominal phasic flexor muscle contraction that jerks the crayfish rapidly backward. The lateral giants respond to caudally located stimuli and evoke a pattern of muscular contraction that thrusts the abdomen upward in the start of a forward somersault (*4, 5*).

The I3 of each side has its soma and dendrites in the third abdominal ganglion, where it is strongly excited by medial and lateral giants bilaterally; its axon projects to the last abdominal (sixth) ganglion (*3*). When the I3's are directly stimulated to fire bilaterally, the most conspicuous consequence is contraction of the ventral and posterior telson flexors, two tail fan muscles innervated by motor nerves of the last ganglion. Often a single I3 suffices to produce the same effects. These contractions are the result of apparently monosynaptic connections between I3 and posterior and ventral telson flexor motoneurons in the last ganglion (*3*).

These connections were surprising, because in healthy preparations firing of the lateral giants always recruits both I3's (*3*), yet it has been reported that the posterior and ventral telson flexor muscles do not contract during lateral giant-mediated tail flips (*5*). Indeed, it is believed that were they to contract, the animal's trajectory would be more backward than upward and the animal would tend to move toward the stimulus it should be evading (*4, 5*). We have confirmed that firing of the I3's alone causes telson flexor muscles to contract, whereas firing of the lateral giants, which

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drives both I3's, does not (Table 1).

Thus, during lateral giant–mediated tail flips, I3 is functionally disconnected from the posterior and ventral telson flexor motoneurons. How this is accomplished is shown in Fig. 1A, which is an intracellular record of the responses of a ventral telson flexor motoneuron to firing of I3 and a lateral giant. In this preparation, an I3 action potential produces a large but subthreshold excitatory postsynaptic potential (EPSP) in the ventral telson flexor motoneuron (panel a in Fig. 1A). When a lateral giant is fired (panel b) it drives I3, which in turn produces its EPSP in the motoneuron on schedule; however, about 1.5 msec before this a depolarizing inhibitory postsynaptic potential (IPSP) (*6*) arises in the motoneuron and the EPSP is consequently attenuated. Thus the lateral giants strongly excite ventral and posterior telson flexor motoneurons via I3 but nullify this excitation by concurrently inhibiting them.

Knowing that the lateral giants reliably recruit I3 yet nullify its most conspicuous motor action, we presumed that the firing of I3 must have other, less overt effects that are synergistic to the production of lateral giant–mediated tail flips. We found that, in addition to exciting posterior and ventral telson flexor motoneurons, I3 monosynaptically but weakly excites most nongiant fast flexor muscle motoneurons in ganglia 4 and 5 and

Table 1. Occurrence of twitch and electrical responses in posterior telson flexors (PTF) and ventral telson flexors (VTF) to single firings of both I3's or of the lateral giant (LG) fibers.

Fibers stimulated	Muscles responding/ muscles tested	
	PTF	VTF
I3's	13/15	7/11
LG's	0/13	0/6

indirectly recruits neurons that inhibit transmission from tail fan afferents to some first-order interneurons (panel a in Fig. 1B) (*3*). The excitation of fast flexor motoneurons is of uncertain significance, since in the fourth and fifth ganglia these motoneurons fire variably and to unknown effect during lateral giant tail flips (*3, 7*). However, the recruitment of afferent inhibition occurs during all giant fiber–mediated escape reactions and is important for canceling reafference from the vigorous tail-flip movement, which would interfere with the behavior (*8*). We presume that it is for its role in producing this and perhaps other unknown effects that I3 is fired during lateral giant–mediated tail flips.

We anticipated that the linkage between I3 and the ventral and posterior telson flexor motoneurons would be expressed and adaptive during medial giant–mediated tail flips, since posterior and ventral telson flexor muscle contractions contribute critically to these. But we found that the medial giants, like the lateral giants, evoke an IPSP in the posterior and ventral telson flexor motoneurons and attenuate excitation from I3 (panel c in Fig. 1A). The medial giants do fire these motoneurons, but through monosynaptic connections, the EPSP's from which peak before the onset of the inhibition (panel c in Fig. 1).

Thus we looked to yet another variety of tail-flip behavior for a role for the connection between I3 and the telson flexor motoneurons. Crayfish tail flips are not always mediated by giant fibers (*9*). When exposed to gradually developing threats, crayfish often escape by means of tail flips that are mediated entirely by nongiant ("voluntary") circuitry (*4*). In contrast to giant fiber–mediated escape reactions, which are very stereotyped in form, tail flips not mediated by giant fibers are variable and move the animal in a wide range of trajectories (*4*). The circuitry that generates these tail flips is uncharted, although it is known that these tail flips, unlike those mediated by giant fibers, cannot be elicited after the head ganglia are removed.

To examine the possible role of I3 and its tail-fan motoneuron connections in nongiant circuitry–mediated tail flips, we prepared animals with essentially intact

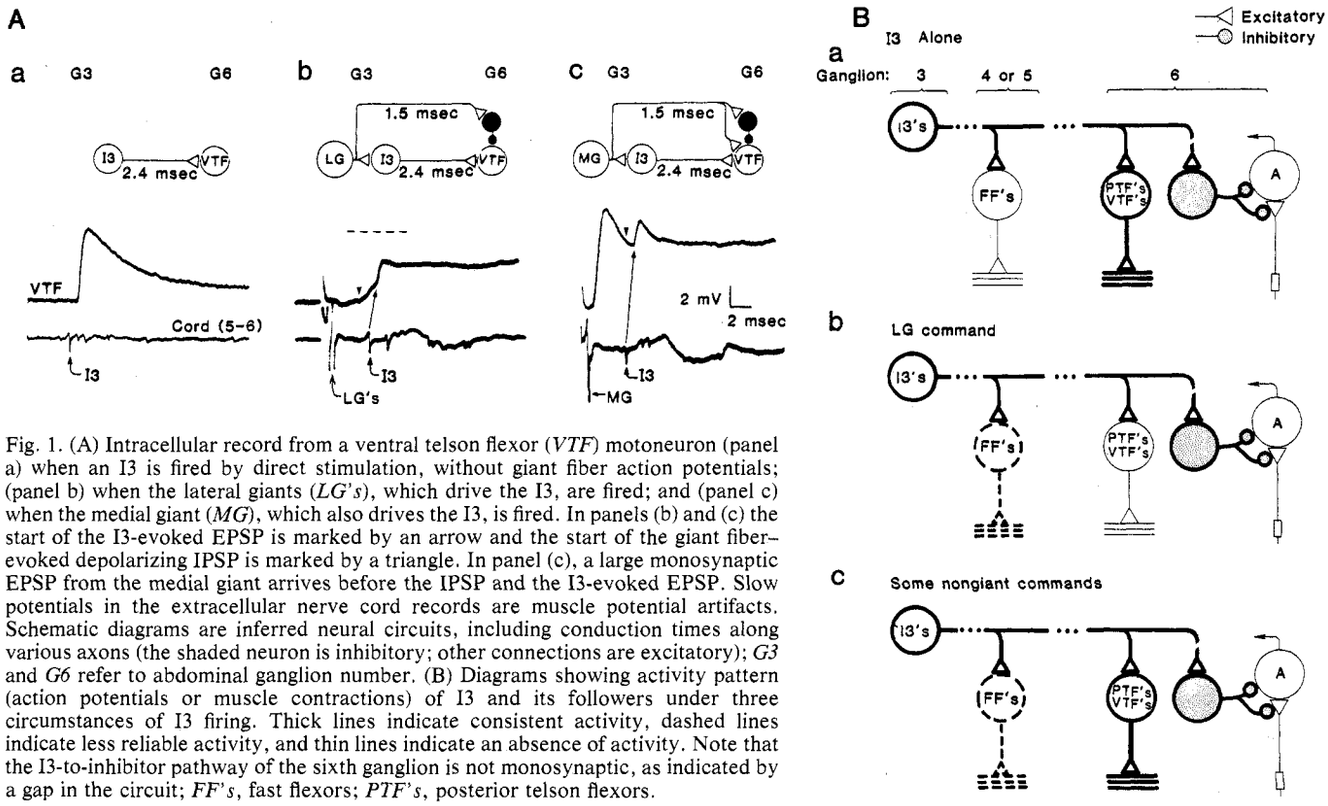

Fig. 1. (A) Intracellular record from a ventral telson flexor (*VTF*) motoneuron (panel a) when an I3 is fired by direct stimulation, without giant fiber action potentials; (panel b) when the lateral giants (*LG's*), which drive the I3, are fired; and (panel c) when the medial giant (*MG*), which also drives the I3, is fired. In panels (b) and (c) the start of the I3-evoked EPSP is marked by an arrow and the start of the giant fiber–evoked depolarizing IPSP is marked by a triangle. In panel (c), a large monosynaptic EPSP from the medial giant arrives before the IPSP and the I3-evoked EPSP. Slow potentials in the extracellular nerve cord records are muscle potential artifacts. Schematic diagrams are inferred neural circuits, including conduction times along various axons (the shaded neuron is inhibitory; other connections are excitatory); *G3* and *G6* refer to abdominal ganglion number. (B) Diagrams showing activity pattern (action potentials or muscle contractions) of I3 and its followers under three circumstances of I3 firing. Thick lines indicate consistent activity, dashed lines indicate less reliable activity, and thin lines indicate an absence of activity. Note that the I3-to-inhibitor pathway of the sixth ganglion is not monosynaptic, as indicated by a gap in the circuit; *FF's*, fast flexors; *PTF's*, posterior telson flexors.

central nervous systems for I3 recording (Fig. 2A) (*10*). Responses mediated by nongiant circuitry usually could be obtained by brushing or pinching the tail fan, brushing the soft cuticle between abdomen and thorax, or pinching more rostral appendages. We found that I3 was always depolarized before and during the responses and fired during 18 percent of them (Fig. 2B). Since the form of nongiant circuitry–mediated tail flips is variable, a given component of the pattern generator would not necessarily fire during every response. In particular, I3 might fire only during responses involving contractions of the posterior or ventral telson flexor muscles. Even then, it would not necessarily always fire, since there might be complementary routes for driving these muscles. In fact, during responses in which neither posterior nor ventral telson flexor muscles contracted, I3 almost never fired, whereas during responses in which they did, I3 fired about 20 percent of the time (median of four preparations).

To determine whether I3 actually produces telson flexor muscle contractions when fired during tail flips mediated by nongiant circuitry, we examined the motor consequences of action potentials evoked by extracellular stimulation of isolated I3 axons during such tail flips. As shown in the inset in Fig. 2C, I3 action potentials that occur during responses mediated by nongiant circuitry are followed closely by posterior telson flexor motoneuron spikes and corresponding muscle potentials. In the histogram this appears as an increased probability of posterior telson flexor motoneuron firing during the interval when these motoneurons should have been depolarized by I3-evoked EPSP's (*11*).

Thus our results indicate that I3 is used in the production of several varieties of tail-flip movements, but the consequences of its firing differ according to the variety of tail flip being generated (Fig. 1B). During nongiant circuitry–mediated tail flips the connection between I3 and posterior and ventral telson flexor motoneurons is functional, and it often fires them. During tail flips mediated by giant fibers, I3 is functionally disconnected from these motoneurons by inhibitory circuitry driven by the giants, but other outputs of I3 remain functional. An I3 interneuron and its targets may thus be considered an example of a basic motor pattern–generating unit that can be called into use and modified in logical structure by higher level controllers.

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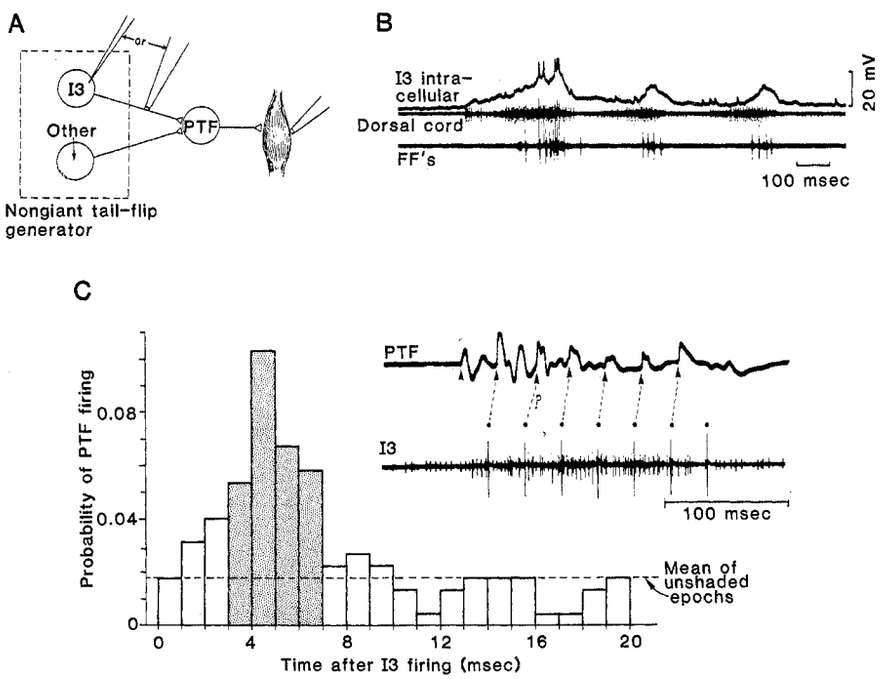

Fig. 2. Experiments with tail-flip responses mediated by nongiant circuitry. (A) Schema of the experimental arrangement. (B) Upper trace, intracellular recording from cell body of I3 during a sequence of three tail flips; middle trace, extracellular recording from a dorsal bundle of nerve cord fibers containing the axon of I3 (the large nerve impulses are from I3); lower trace, extracellular recording from a phasic motor nerve innervating tail-flip flexor muscles of the third abdominal segment [electrode not indicated in (A)]. Each burst of activity is a separate tail-flip response. Note that I3 fired only on the first tail flip of the sequence, but was depolarized during all three tail flips. (C) Probability of posterior telson flexor activity following an I3 action potential during tail flips. The period during which posterior telson flexor motoneurons would be expected to fire as the result of an I3 action potential is shaded. The inset shows data for a typical response. Lower trace, extracellular recording from a cord fiber bundle containing an I3 axon (I3 impulses marked by dots); upper trace, intramuscular recording from the posterior telson flexor muscle (posterior telson flexor motoneuron impulses, too small to see at this gain, are marked by triangles). Each posterior telson flexor motoneuron spike that appears to be caused by a spike in the experimental I3 is indicated by a dashed line. Note that other interneurons can also drive posterior telson flexor muscles and that the stimulated I3 does not always do so (*11*).

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10. Before dissection blood was replaced by ice-cold, oxygen-saturated crayfish Ringer solution to prevent embolisms, and during dissection all arteries that were cut were tied off proximal to the heart to maintain blood pressure.
11. The probability of a posterior telson flexor motoneuron firing in response to an I3 action potential was presumably reduced by residual refractoriness from prior firing caused either by the immediately preceding I3 action potential or by the animal's own motor program; conversely, the responses of these motoneurons to the animal's own input was reduced by prior action potentials caused by our stimulation.
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