pressure mercury bulb (HBO 100 W/2, Osram) was focused onto an aperture diaphragm imaged at the back focal plane of the objective (Zeiss Luminar 40 mm; numerical aperture, 0.13). This produced a luminous disk pattern imaged at infinity, subtending 7° of arc and having an intensity about three decades above the threshold for pigment granule migration (*8*). The fly observed this pattern for 30 minutes, with the dorsal-frontal part of its retina. Fluorescence increase in the photoreceptors was monitored by continuously observing the deep pseudopupil (*3*).
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Identified Interneurons Produce Both Primary Afferent Depolarization and Presynaptic Inhibition

Abstract. *Crayfish interneurons were identified that appear to be directly responsible for presynaptic inhibition of primary afferent synapses during crayfish escape behavior. The interneurons are fired by a polysynaptic pathway triggered by the giant escape command axons. When directly stimulated, these interneurons produce short-latency, chloride-dependent primary afferent depolarizations and presynaptically inhibit primary afferent input to mechanosensory interneurons.*

Presynaptic inhibition decreases synaptic efficacy by reducing the release of transmitter substances from presynaptic terminals (*1, 2*). In the central nervous system of invertebrates and vertebrates, presynaptic inhibition of the release of transmitters in primary afferent fibers is correlated with primary afferent depolarization (PAD) (*3, 4*). The mechanisms for PAD and presynaptic inhibition in the central nervous system have not been determined, in part because the interneuronal pathways effecting PAD are unknown (*5*). We have studied the polysynaptic pathway by which the giant, escape command axons of the crayfish inhibit transmitter release in primary mechanosensory afferents. The synapses of these afferents are depression-prone and are responsible for behavioral habituation of the escape tail-flip (*6*). The pathway producing presynaptic inhibition is of special interest because it protects the afferent synapses from the depression that would result as a consequence of reafference during the tail-flip (*7*), and thus prevents habituation of the escape response caused by the animal's own movement. We have identified what appear to be the final inhibitory interneurons for command-derived presynaptic inhibition. These newly identified cells are fired by the giant escape command neurons and produce both PAD in mechanosensory afferents and presynaptic inhibition of primary afferent input to first-order mechanosensory interneurons.

Our experimental preparation was the isolated abdominal nerve cord of the crayfish *Procambarus clarkii* (*8*). Simultaneous intracellular recordings were obtained from interneurons and primary afferent axons in the neuropil of the sixth abdominal ganglion. Many of the primary afferent axons are of large diameter (6 to 30 μm) and can be routinely impaled with intracellular electrodes (*9*). With intracellular dye injections we determined that the afferents terminate within 300 μm of the margin of the neuropil after entering the sixth ganglion. Our electrodes were positioned in the afferent axons near the point where they entered the neuropil and thus were close to the sites of PAD production. This is supported by the fact that the amplitude of PAD could be altered by imposed polarization of the afferent membrane as well as intracellular chloride injection. In several experiments a sucrose gap recording of PAD from the fifth sensory root was combined with intracellular impalements of a PAD-producing inhibitory interneuron (PADI) in the sixth ganglion and an identified primary sensory interneuron. A simplified diagram of the pathways studied and the sites of recording and stimulation is presented in Fig. 1A.

To be classified as a PADI, a cell had to produce short-latency PAD and be fired by a single giant axon impulse. We studied a total of 34 PADI's in 31 animals. A single action potential in a giant escape command axon results in presynaptic inhibition of primary afferent synapses (*7*). Cells identified as PADI's always fired, and sometimes gave multiple spikes, after a single giant axon impulse (Fig. 1B). The time from the giant axon impulse to the first spike in a PADI was 11.4 ± 4.6 msec (mean ± standard deviation). Several lines of evidence (*10*) indicate that the pathway from the giant axons to the PADI's is polysynaptic and involves interganglionic interneurons. PADI's were never fired by stimulation of any of the sensory roots at stimulus intensities below the threshold of the lateral giant escape command axons (*11*).

When PADI's were fired directly by the injection of depolarizing current, their spikes produced constant PAD's with a mean latency of 0.97 ± 0.12 msec (n = 15) and a mean duration of 36.2 ± 9.2 msec (n = 15). These unitary PAD's were capable of following PADI impulses one-for-one at frequencies greater than 100 Hz, and their amplitude was increased by intracellular injection of chloride. Another notable aspect of the unitary PAD's was that their amplitude fluctuated in discrete steps (Fig. 1C). We have not analyzed these fluctuations, but the short, constant latency makes it highly unlikely that they are caused by the intermittent firing of an interposed interneuron.

In the seven cases tested, directly elicited PADI impulses produced both PAD and presynaptic inhibition of primary afferent input to identified sensory interneurons. An example from one experiment is presented in Fig. 1, D to G. Primary afferents were stimulated by

shocking a sensory root with a stimulus strength just sufficient to consistently produce a single impulse in the postsynaptic interneuron (Fig. 1D) (*12*). When the afferent volley was preceded by a pair of directly elicited spikes in the PADI, the excitatory postsynaptic potential (EPSP) in the interneuron was reduced in amplitude to a level subthreshold for impulse production (Fig. 1E). PAD was recorded with a sucrose gap on the fifth root (top trace in Fig. 1E). The reduction in EPSP amplitude was maximal when the peak of the unitary PAD's coincided with the afferent root shock.

The PADI's produced no detectable inhibitory postsynaptic potentials (IPSP's) in the mechanosensory interneurons (*13*), and the existence of a remote increase in postsynaptic conductance was ruled out by the following observations. First, there was no obvious change in the time course of subthreshold, evoked EPSP's in the sensory interneurons when paired with PADI spikes at latencies producing maximal inhibition. Second, the amplitude of antidromic spikes in the sensory interneurons was not influenced by concurrent firing of the PADI's. Finally, impulses in the sensory interneuron elicited directly by depolarizing current were not influenced by concomitant driving of the PADI's (Fig. 1, F and G). Thus we conclude that inhibition of the mechanosensory interneurons by the PADI's was entirely presynaptic.

The structure (*14*) of the PADI's is consistent with their physiologically determined connections (Fig. 2). We have seen three morphological types of PADI. All three have unilateral dendrites (*15*) located in the dorsal part of the neuropil in the anterior part of the ganglion, a region that also contains axonal terminals of interganglionic interneurons fired by the giant axons. The PADI axonal branches (*15*) and their varicose terminals are bilateral and ramify extensively within the ventral innervation fields of all ten sensory roots of the sixth ganglion (*16*). One type of PADI (Fig. 2) has a very fine axon in the ventromedial region of the nerve cord ipsilateral to its dendrites. Its cell body is also located ipsilateral to its dendrites in the rostral soma cluster (*17*); these neurons may be responsible for the inhibition and short-latency PAD that can be produced by

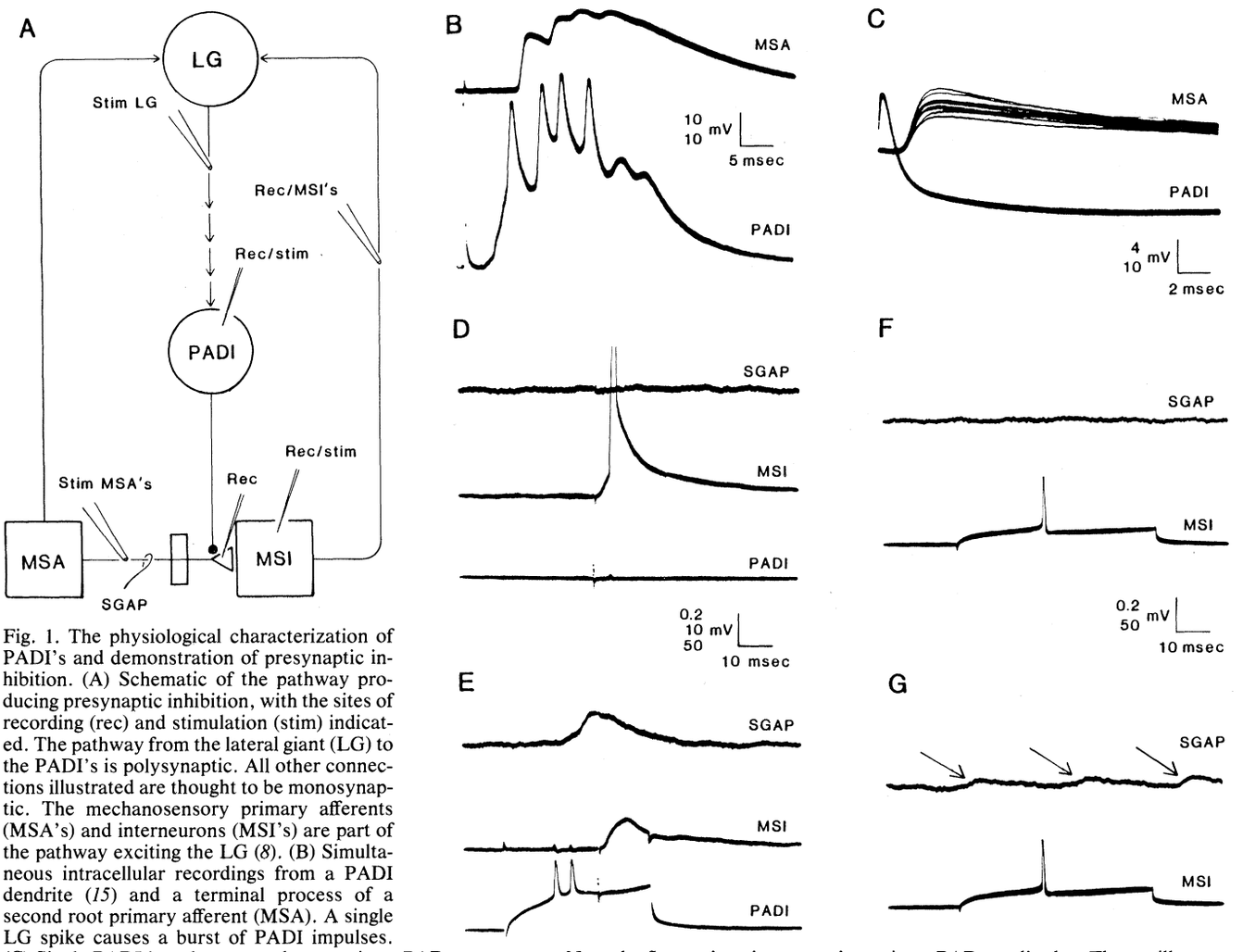

Fig. 1. The physiological characterization of PADI's and demonstration of presynaptic inhibition. (A) Schematic of the pathway producing presynaptic inhibition, with the sites of recording (rec) and stimulation (stim) indicated. The pathway from the lateral giant (LG) to the PADI's is polysynaptic. All other connections illustrated are thought to be monosynaptic. The mechanosensory primary afferents (MSA's) and interneurons (MSI's) are part of the pathway exciting the LG (*8*). (B) Simultaneous intracellular recordings from a PADI dendrite (*15*) and a terminal process of a second root primary afferent (MSA). A single LG spike causes a burst of PADI impulses. (C) Single PADI impulses cause large, unitary PAD components. Note the fluctuations in successive unitary PAD amplitudes. The oscilloscope was triggered from the rising phase of the PADI spikes and several responses were superimposed. The amplitude of unitary PAD was increased by intracellular chloride injection. (D) A single shock to the second sensory root fires the impaled sensory interneuron (MSI), physiologically identified as 6C1 (*17*). (E) Presynaptic inhibition of primary afferent input to 6C1. The second root shock was preceded by a pair of PADI spikes elicited directly by intracellular current injection, and the EPSP in 6C1 is reduced to subthreshold for spike production. Note that the peak PAD, seen in the sucrose gap recording (SGAP) of the fifth sensory root, coincides with the second root shock. (F and G) Remote postsynaptic conductance increases do not occur after PADI impulses. (F) A constant-current pulse was injected into the MSI (6C1) and directly elicited a single action potential. (G) The PADI was concurrently driven by a step of injected current (trace not shown) and produced repetitive unitary PAD as recorded in the sucrose gap (arrows). However, the impulse in 6C1 continued to occur, and at the same time with respect to the onset of the current pulse.

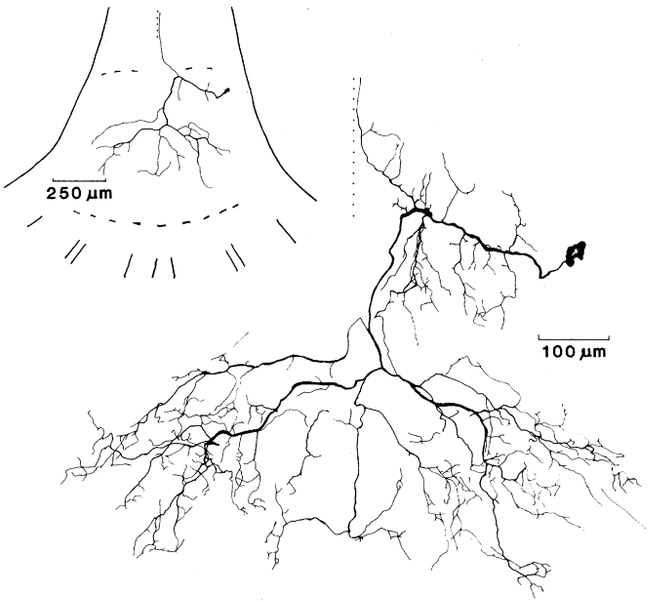

Fig. 2. Structure of a PADI (*14*). Inset: low-magnification view showing the outlines of the sixth abdominal ganglion. Vertical dotted lines indicate the midline of the nerve cord.

stimulation of axon bundles in the rostral nerve cord (*18*). In three preparations, we dye-filled two such interneurons with highly overlapping processes and juxtaposed cell bodies. In all cases at least one of the filled cells was physiologically identified as a PADI, and in one case each cell was shown to be a PADI.

Another type of PADI ($n = 4$) was nearly identical to that described above, but lacked an axon in the nerve cord (that is, it was a "local" neuron). A third type, ($n = 3$), also local, had its soma in the caudal cell body cluster (*17*). The three morphological types were physiologically indistinguishable. Thus we have evidence for three structurally distinguishable PADI's that cause equivalent and additive PAD; the examples of two cells with highly overlapping processes suggest that each cell type may represent a small population of similar neurons. Since the PADI's have bilateral axonal branches, our results predict a minimum of eight PADI inputs to an afferent, assuming that the PADI's are bilaterally paired and have completely overlapping connectivity. In support of this prediction, unitary PAD produced by an individual PADI has been recorded successively in first-, third-, fourth-, and fifth-root afferent terminals. In addition, afferents in any given sensory root that receive giant axon–evoked PAD have invariably received unitary PAD from the simultaneously impaled PADI.

In an effort to determine the number of PADI's impinging on a given primary afferent, we hyperpolarized an impaled PADI and prevented it from firing after a giant axon stimulus. However, because PADI's fire multiply and unitary components of PAD are large, a relatively small number of inputs can cause PAD to plateau at the reversal potential. These factors prevented us from estimating the total number of PADI's in the pathway.

We conclude that, in this system, presynaptic inhibition is effected by a population of inhibitory interneurons, each of which synapses on a great many afferent terminals. We have shown that a single PADI produces both PAD and presynaptic inhibition. Preliminary evidence suggests that the inhibitory interneurons release γ-aminobutyric acid (GABA) and cause PAD by increasing the chloride conductance of the afferent terminals (*18, 19*). We cannot absolutely exclude a role for extracellular potassium accumulation in the production of PAD, but any such role must act in concert with the synaptic effects.

Afferent axons in the crayfish have large diameters and sparse terminal branching, and single afferents produce easily recorded, unitary EPSP's in identified sensory interneurons (*8*). Therefore, it is feasible in this system to test directly the contributions of depolarization, shunt conductance increase (*1, 3, 4, 20*), and calcium conductance decrease (*2, 21*) to the production of presynaptic inhibition. The mechanisms of presynaptic inhibition described here may also apply to the vertebrate central nervous system, since, in both crayfish and vertebrates, presynaptic inhibition of primary afferent release is of long duration and is associated with PAD that is attributed to a GABA-mediated increase in chloride conductance.

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9. PADI penetrations were made with glass microelectrodes containing 3 percent Lucifer yellow-CH [W. W. Stewart, *Cell* **14**, 741 (1978)] in the tip and backfilled with 2.5*M* LiCl (impedance, 130 to 300 megohms). Primary afferent terminals were impaled with microelectrodes containing 3*M* KCl (impedance, 15 to 30 megohms) or Lucifer yellow in order to obtain the terminal morphology. Suction electrodes were used for extracellular recording and stimulation. The lateral and medial giant axons were selectively stimulated after placement of the suction electrode on the dorsal surface of the nerve cord between the first and second ganglia.
10. M. D. Kirk, in preparation.
11. Sensory root shocks that were suprathreshold for sensory interneuron discharges (monitored in the nerve cord between the fifth and sixth ganglia) never excited an impaled PADI above spike threshold, even though PAD was elicited by the same stimulus. Also, we impaled and dye-filled a different local sensory interneuron that was fired by sensory root shock and produced constant, short-latency PAD. This local interneuron received only subthreshold input after a giant axon stimulus.
12. The postsynaptic interneuron in this case was physiologically identified as interneuron C (*8*), also named 6C1 (*17*). This interneuron is part of the afferent circuit that excites the lateral giant command cells (*8*) and does not receive postsynaptic inhibition after giant axon firing (*17*).
13. Interneuron A or 6B1 (*17*), which is also part of the afferent circuit that excites the lateral giants (*8*), is postsynaptically inhibited after the firing of a giant axon (*7*). Some PADI's produced short-latency IPSP's in interneuron A while others apparently did not. When there was no detectable IPSP in interneuron A from the impaled PADI, the PADI still produced inhibition of primary afferent input.
14. Physiologically identified PADI's were injected with Lucifer yellow and the ganglia were fixed overnight in phosphate-buffered 10 percent Formalin. After alcoholic dehydration and clearing in methyl salicylate, whole mounts were photographed with Kodak slide film (400 ASA). The PADI's were traced from projections of the slides taken at a series of different focal planes.
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