Convergent Chemical and Electrical Synaptic Inputs From Proprioceptive Afferents Onto an Identified Intersegmental Interneuron in the Crayfish

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Nagayama, Toshiki, Hitoshi Aonuma, and Philip L. Newland. 1993. Synaptic inputs to the intersegmental interneurons that excite LG, however, are chemically mediated and depression prone, but protected from this depression to some extent by presynaptic inhibitory pathways (Kennedy et al. 1974; Kirk and Wine 1984; Newland et al. 1996). These intersegmental interneurons in turn excite LG via electrical synapses (Zucker 1972). The combined mono- and disynaptic electrical inputs from sensory afferents onto LG sum to evoke spikes and thus initiate the tail-flip escape response.

A proprioceptor, the exopodite-endopodite chordotonal organ, containing 12 sensory neurons that project to the terminal abdominal ganglion (Nagayama and Newland 1993), monitors the movements of the exopodite relative to water displacements (Wine and Krasne 1982). Their sensory neurons may excite LG either monosynaptically or disynaptically via a specific set of ascending intersegmental interneurons (Zucker et al. 1971). Although it was thought for many years that this input to LG was exclusively via electrical synapses, recently it has been shown that the monosynaptic inputs to LG are probably mediated by both electrical and chemical transmission (Miller et al. 1992; Yeh et al. 1993).
the joint between the endopodite and exopodite (Field et al. 1990). An oil hook electrode was placed on nerve 3 between the protopodite and ganglion to monitor the activity of sensory neurons innervating the chordotonal organ. The nerve was cut distal to the chordotonal organ. The chordotonal organ was mechanically stimulated by displacing its strand with the use of a pin mounted on a precision vibrator (Ling Altec type 101). A full description of the methods is given in Nagayama and Newland (1993).

For intracellular recording, the ganglion was treated with protease (Sigma type XIV) for 30 s to aid penetration of microelectrodes filled with 3% Lucifer yellow CH (Stewart 1978) dissolved in 0.1 M lithium chloride. Electrodes had resistances of ~80–150 MΩ. After physiological characterization, interneurons were stained by injecting them with Lucifer yellow, and the ganglia containing them were dissected from the crayfish and their identity confirmed under a fluorescence microscope (Olympus). All recordings were stored on a PCM data recorder for subsequent analysis and displayed on a Tektronix digital oscilloscope. Data were transferred from the oscilloscope to a computer running Lab Windows software (National Instrument) with the use of a GPIB board. Data are based on 14 successful recordings of interneuron A.

**RESULTS**

Displacing the strand of the chordotonal organ evoked a burst of spikes in its sensory neurons and a barrage of depolarizing potentials in interneuron A (Fig. 1A). Superimposed sweeps of an oscilloscope triggered by spikes in different sensory neurons, selected with the use of a window discriminator, showed that EPSPs followed sensory spikes with two distinctly different, but constant, latencies (Fig. 1B) \( t = -12.77, P < 0.01, 4 \text{ df}; \text{ Student's paired } t\text{-test} \). The first, triggered from large amplitude extracellular sensory spikes, had short latencies of 1.48 ± 0.19 (SE) ms \((n = 5 \text{ crayfish})\) and were of large amplitude \((1–2 \text{ mV})\) (Fig. 1, Bi and Bii). The second, triggered from small-amplitude extracellular spikes, had longer latencies of 2.5 ± 0.17 ms \((n = 5 \text{ crayfish})\) and were smaller in amplitude \((0.5 \text{ mV})\) (Fig. 1Biii).

The activity of sensory neurons innervating the chordotonal organ was monitored from the nerve 3.8 ± 0.38 (SE)
mm from the synaptic sites of interneuron A in the ganglion. Because the sensory neurons of the chordotonal organ have rapid conduction velocities with a mean of $3.1 \pm 1.2 \text{ m/s}$ (Nagayama and Newland 1993), a conduction time of almost 1.0 ms would be needed to convey the sensory spikes to the synaptic terminals. For the short-latency potentials this mean conduction velocity would leave no time for the synaptic delay of conventional chemical synaptic transmission. This suggests that either rapidly conducting afferents mediate transmission of these short latency EPSPs in interneuron A, or transmission is via electrical synapses. On the other hand, central delays of 1–1.5 ms would account for the longer latency inputs onto interneuron A, implying that they could be mediated by chemical synaptic transmission from slowly conducting afferents.

To resolve this issue, evidence for electrical and chemical synaptic inputs onto interneuron A was sought with the use of three tests. First, continuous hyperpolarizing current was injected into interneuron A while the chordotonal organ was stimulated mechanically. The amplitudes of the short-latency EPSPs were unaltered by the accompanying changes in membrane potential (Fig. 2A). Such a lack of change is characteristic of many electrical synapses. By contrast, the amplitudes of the longer-latency EPSPs were increased with hyperpolarizing current, so that they were up to 50% greater with $-2 \text{nA}$ (Fig. 2B). A change of this nature is characteristic of chemical transmission.

Second, bathing the nervous system in a saline containing mechanosensory neurons so far investigated use acetylcholine as their transmitter (Barker et al. 1972; Casagrande and Ritzman 1992; Leitch and Pitman 1995; Miller et al. 1992; Ushizawa et al. 1996), and although it is likely that these chordotonal afferents also use acetylcholine, we have not yet carried out a detailed study of the pharmacology of transmission. None of the characteristic features of either type of synapse, on their own, provide conclusive evidence for one form of synaptic transmission or the other. For example, Zucker et al. 1972; Zucker et al. 1971, and bath in curare, an antagonist of cholinergic nicotinic transmission. All of these features are characteristic of the short latency potentials from chordotonal afferents to interneuron A. Thus the EPSPs from chordotonal afferents onto interneuron A are likely to be mediated by electrical transmission. The longer latency potentials in interneuron A, however, are typical of chemical synaptic inputs, and were dramatically altered by current injection (Burrows and Pfütger 1988; Nagayama and Sato 1993; Zucker 1972), reduced in low Ca$^{2+}/$saline (Parker and Newland 1995), and reduced by bath application of curare, typical of cholinergic transmission (Miller et al. 1992; Ushizawa et al. 1996). All insect and crustacean mechanosensory neurons use acetylcholine as their transmitter (Barker et al. 1972; Casagrande and Ritzman 1992; Leitch and Pitman 1995; Miller et al. 1992; Parker and Newland 1995; Trimmer and Weeks 1989; Ushizawa et al. 1996), and although it is likely that these chordotonal afferents also use acetylcholine, we have not yet carried out a detailed study of the pharmacology of transmission.

Third, bath application of 1 mM curare ($n = 5$), a nicotinic antagonist, likewise had no effect on the short latency EPSPs in interneuron A even after 34 min of bath application (Fig. 3C), but again reduced reversibly the amplitude of the longer latency EPSPs (Fig. 3D).

**DISCUSSION**

We show that an identified intersegmental interneuron, involved in the activation of the LGs in the crayfish escape network, receives both direct electrical and chemical synaptic inputs in the form of short and long latency inputs from sensory neurons innervating a proprioceptor in the tailfan. EPSPs mediated by electrical transmission at other crayfish synapses have no central synaptic delay and are in many cases unchanged by depolarizing or hyperpolarizing current injection, bathing in low Ca$^{2+}/$high-Mg$^{2+}$ saline (Zucker 1972; Zucker et al. 1971), or bathing in curare, an antagonist of cholinergic nicotinic transmission. All of these features are characteristic of the short latency potentials from chordotonal afferents to interneuron A. Thus the EPSPs from chordotonal afferents onto interneuron A are likely to be mediated by electrical transmission. The longer latency potentials in interneuron A, however, are typical of chemical synaptic inputs, and were dramatically altered by current injection (Burrows and Pfütger 1988; Nagayama and Sato 1993; Zucker 1972), reduced in low Ca$^{2+}$ saline (Parker and Newland 1995), and reduced by bath application of curare, typical of cholinergic transmission (Miller et al. 1992; Ushizawa et al. 1996).
al. (1971) showed that for some chemical synapses in the crayfish bath application of low Ca$^{2+}$ had little effect on the postsynaptic potential. Moreover, electrical synapses between primary afferents and LGs are voltage sensitive (Edwards et al. 1991), whereas those described here were unaffected by current injection, although this may be due to the fact that we were able to inject only small hyperpolarizing currents into interneuron A. Taken together, however, all of the features we describe for these synapses provide strong evidence for the convergence of chemical and electrical synaptic inputs from different proprioceptive afferents onto interneuron A.

Only recently has it been suggested that LG interneurons, because they possess nicotinic receptors, receive chemical as well as electrical synaptic inputs from water-motion-sensitive afferents (Miller et al. 1992), although it had been suggested that direct chemical transmission from primary afferents did not normally occur. However, in a brief report Yeh et al. (1993) showed that LG does in fact receive input mediated by chemical transmission from water-motion-sensitive afferents. Likewise we now show that interneuron A, which itself excites LGs through rectifying electrical synapses (Edwards et al. 1991; Zucker 1972), also receives both electrical and chemical inputs from a proprioceptor. In our previous experiments (Newland and Nagayama 1993) we demonstrated chemical synaptic inputs onto interneuron A from the same chordotonal afferents. We failed, however, to note the presence of electrical inputs, but this may have been due to a number of reasons. For example, the stimulus waveforms, peak displacements, and peak velocities were all different between the two studies. Here we used greater displacement angles and higher velocities of stimulation because they possess nicotinic receptors, receive chemical as well as electrical synaptic inputs from water-motion-sensitive afferents (Miller et al. 1992), although it had been suggested that direct chemical transmission from primary afferents did not normally occur. However, in a brief report Yeh et al. (1993) showed that LG does in fact receive input mediated by chemical transmission from water-motion-sensitive afferents. Likewise we now show that interneuron A, which itself excites LGs through rectifying electrical synapses (Edwards et al. 1991; Zucker 1972), also receives both electrical and chemical inputs from a proprioceptor. In our previous experiments (Newland and Nagayama 1993) we demonstrated chemical synaptic inputs onto interneuron A from the same chordotonal afferents. We failed, however, to note the presence of electrical inputs, but this may have been due to a number of reasons. For example, the stimulus waveforms, peak displacements, and peak velocities were all different between the two studies. Here we used greater displacement angles and higher velocities of stimulation of the chordotonal organ, which could account for afferents with electrical outputs being recruited that were silent in previous experiments. The results presented here, however, do suggest that there are two populations of chordotonal
afferents, one making electrical synapses with interneuron A, the other making chemical synapses. Further studies are now needed to determine which chordotonal afferents have chemical outputs and which have electrical ones. Moreover, are the output properties of an afferent dependent on their coding properties?

The fact that interneuron A receives electrical synaptic input from proprioceptive afferents adds to what has been described to date for this interneuron (Zucker 1972; Zucker et al. 1971). Most previous studies of interneurons in the escape circuit exciting LG have concentrated on water motion input on to a number of interneurons in the disynaptic pathway, especially interneuron A (Bryan and Krasne 1977; Krasne 1969; Nagayama and Sato 1993; Nagayama et al. 1993; Zucker 1972). The synapses from these hair afferents have been regarded as being crucial in the exteroceptive input pathway to LG, because they probably act as sites of habituation to repetitive stimulation (Krasne 1969) and thus provide the neuronal pathways necessary for behavioral plasticity. Our finding that interneuron A also receives electrical inputs from chordotonal afferents raises a further crucial question as to why both types of synaptic transmission are necessary at this synapse. Presumably this dual mode of transmission allows more plasticity than electrical transmission alone can provide, but also with the additional benefit of speed of transmission in a neuronal circuit in which rapid transmission is paramount to produce the short latency escape behavior. Clearly, then, different modalities of sensory input have different influences on interneuron A. We must now look at other interneurons in the di- and polysynaptic pathways exciting LG to analyze their responses to proprioceptive input, and moreover attempt to understand the functional consequences for dual synaptic inputs on the circuits mediating escape behavior.

We are grateful to Prof. Masakazu Takahata for making available all laboratory facilities during the course of this work in Hokkaido University, and to M. Burrows, T. Friedel, T. Matheson, O. Morris, and M. Wildman in Cambridge for comments on earlier drafts of this manuscript.

P. L. Newland was supported by a Royal Society study visit grant, an Advanced Fellowship from the Biotechnology and Biological Sciences Research Council (United Kingdom), and a Wellcome Trust grant to Malcolm Burrows. T. Nagayama was supported by Ministry of Education, Science, Sport and Culture Grant 08640856. on an identification motoneurone and its direct afferent inputs in larval Procambarus clarkii. J. Comp. Physiol. 76: 1047–1058, 1996.


