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

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INTRODUCTION

Electrical synapses are especially prevalent in neural circuits where speed and reliability of transmission are important. Thus it is not surprising to find electrical synapses in circuity mediating rapid escape movements, both in invertebrates such as crayfish (Wine 1984; Wine and Krasne 1982) and in vertebrates such as teleost fish (Eaton and Hacket 1984).

The escape movements, or tail-flips, of crayfish consist of short latency rapid flexions of the abdominal segments that propel the animal away from a threatening stimulus. Tail-flips are mediated by giant interneurons, one pair of which, the lateral giant interneurons (LGs), receives inputs from sensory exteroceptors on the tailfan that respond 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). 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 the abdominal ganglion (Field et al. 1990). One particular intersegmental ascending interneuron that excites LG, called interneuron A (Zucker 1972) or NE-1 (Nagayama et al. 1993), receives input not only from exteroceptive water-motion-sensitive hairs but also from sensory neurons of this chordotonal organ (Newland and Nagayama 1993). These proprioceptive inputs were previously thought to be monosynaptic and chemically mediated. Given that dual modes of transmission (chemical and electrical) are now thought to be involved in the activation of LG, we have analyzed the mode of transmission of proprioceptive inputs to interneuron A. We show that spikes in different proprioceptive afferents are followed at two different latencies by excitatory postsynaptic potentials (EPSPs) in interneuron A that are likely to be mediated by monosynaptic electrical and chemical transmission.

METHODS

The abdomen was removed from adult crayfish (Procambarus clarkii; Girard) and pinned ventral side up in a small chamber (10 ml) containing cooled physiological saline (van Harreveld 1936). The chamber was constantly perfused with fresh saline at a rate of 4 ml/min with the use of an Eyela micro tube pump (MP-3). The bathing solution could be changed so that a saline with a low calcium concentration (2.7 mM, compared with 13.5 mM in normal saline) or one containing 1 mM curare (d-tubocurarine, Sigma; dissolved in normal saline) could be applied. The terminal (sixth) abdominal ganglion was exposed by removing the fifth sternite and surrounding soft cuticle, and was supported on a silver platform. The exopodite-endopodite chordotonal organ was exposed by cutting a window in a medial region of the protopodite to expose nerve 3 from the terminal ganglion, and then extending the window distally to the endopodite to reveal the chordotonal organ spanning...
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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 df; Student’s paired t-test). The first, triggered from large amplitude extracellular sensory spikes, had short latencies of 1.48 ± 0.19 (SE) ms (n = 5 crayfish) and were of large amplitude (1–2 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 crayfish) and were smaller in amplitude (∼0.5 mV) (Fig. 1Biii).

The activity of sensory neurons innervating the chordotonal organ was monitored from the nerve 3.8 ± 0.38 (SE)

![Diagram of interneuron A](image)

**FIG. 1.** Interneuron A receives proprioceptive input from a chordotonal organ (CO) in the tailfan. A: displacing the chordotonal organ with a ramp stimulus of 4/s and peak amplitude of 10” evoked a burst of chordotonal organ sensory spikes in nerve 3 and synaptic potentials in interneuron A. Bi–Biii: single sweeps of the oscilloscope triggered by chordotonal afferent spikes of 3 different amplitudes, selected by means of a window discriminator. The afferents in Bi and Bii elicited very short, but constant latency (1 ms) potentials in the interneuron. Biii: a 3rd afferent elicited a constant latency potential but with twice the delay (2 ms). Bi: 2 traces superimposed. Bii: 4 traces superimposed. Biii: 3 traces superimposed. Asterisks: subsequent afferent spikes that also give rise to excitatory postsynaptic potentials (EPSPs) in interneuron A. Dashed vertical lines: peaks of sensory spikes and onset of depolarization in interneuron. Data from 1 animal.
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 ± 1.2 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 nA (Fig. 2B). A change of this nature is characteristic of chemical transmission.

Second, bathing the nervous system in a saline containing a low calcium concentration of 2.7 mM (n = 5) again had no effect on the short latency EPSPs (Fig. 3A), characteristic of many electrical synapses (Zucker et al. 1971), but almost abolished the longer latency EPSP after 9 min (Fig. 3B). A reduction in EPSP amplitude in a low concentration of Ca²⁺ is again a feature of chemical 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 Ca²⁺/high-Mg²⁺ 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 Ca²⁺ 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 so far investigated use acetylcholine as their transmitter (Barker et al. 1972; Casagrand 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.

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. (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 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

FIG. 3. Responses of interneuron A to low Ca\(^{2+}\) saline, and to curare. A: responses of interneuron in 2.7 mM Ca\(^{2+}\) saline. Amplitude of short-latency inputs is not affected by bathing the nervous system for 9 min in low Ca\(^{2+}\) saline. Four superimposed sweeps triggered from the afferent spike are shown. B: bath application of low Ca\(^{2+}\) saline gradually reduced the amplitude of a longer latency potential triggered from an afferent spike, until it was <20% of the control after 9 min. Four superimposed sweeps are shown for each trace. C: effects of 1 mM d-tubocurarine, a nicotinic antagonist, on EPSPs in interneuron A. Bath application of 1 mM d-tubocurarine for 34 min had no effect on the short latency potentials in the interneuron. D: longer latency potentials were reduced by over 50% after 30 min but recovered in amplitude after wash in normal saline (t = 34 min). Each trace in C and D is a signal average of 32 sweeps. A and B are from 1 animal and C and D are from another.
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.

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