Synaptic Interactions Between Crista Hair Cells in the Statocyst of the Squid Alloteuthis subulata

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Chrachri, Abdesslam and Roddy Williamson. Synaptic interactions between crista hair cells in the statocyst of the squid Alloteuthis subulata. J. Neurophysiol. 80: 656 ± 666, 1998. Intracellular injections of the fluorescent dye Lucifer yellow into the various cell types within the anterior transverse crista segment of the statocyst of squid revealed that the primary sensory hair cells and both large and small first-order afferent neurons have relatively simple morphologies, each cell having a single, unbranched axon that passes directly into the small crista nerve that innervates the anterior transverse crista. However, the small first-order neurons have short dendritic processes occurring in the region of the sensory hair cells. The secondary sensory hair cells have no centripetal axons, but some have long processes extending from their bases along the segment. Simultaneous intracellular recordings from pairs of the different cell types in the anterior transverse crista segment demonstrated that electrical coupling is widespread; secondary sensory hair cells are coupled electrically along a hair cell row, as are groups of primary sensory hair cells. Secondary sensory hair cell also are coupled to neighboring small first-order afferent neurons. However, this coupling is rectifying in that it only occurs from secondary sensory hair cells to first-order afferent neurons. Direct electrical stimulation of the small crista nerve to excite the efferent axons revealed efferent connections to both the primary sensory hair cells and the small first-order afferent neurons. These efferent responses were of three types: excitatory or inhibitory postsynaptic potentials and excitative postsynaptic potentials followed by inhibitory postsynaptic potentials. The functional significance of the cell interactions within the crista epithelium of the statocyst of squid is discussed and comparisons drawn with the balance organs of other animals.

INTRODUCTION

Cephalopods, such as squids, cuttlefishes, and octopuses, show a well-developed control of balance, maintaining good postural control during both rapid movements, such as jet propelled swimming, and during slow movements, such as precise hovering maneuvers. Many authors have implicated the statocysts in this control of balance, and this has been supported by a number of morphological and physiological studies (Budelmann 1970, 1977; Budelmann et al. 1987a,b; Klein 1932; Williamson 1990; Williamson and Budelmann 1985; Young 1960).

Cephalopods have two statocysts and, with the exception of Nautilus, each of these contains two separate receptor systems: one, the macula/statolith (statoconia) system, is responsible for the detection of linear accelerations, including gravity, whereas the other, the crista/cupula system, is used for detecting angular accelerations. In squids and cuttlefishes, the gravity receptor system is composed of three separate sensory epithelia (maculae): the macula statica princeps (MSP), the macula neglecta superior (MNS), and the macula neglecta inferior (MNI). All three maculae are arranged roughly at right angles to each other (Budelmann 1979; Budelmann et al. 1973; Stephens and Young 1982). The MSP is covered with a statolith, whereas the other two (MNS and MNI) are covered with small statoconia (Budelmann 1977, 1990; Stephens and Young 1982). The squid and cuttlefish angular acceleration receptor system comprises a thin strip of sensory hair cells and associated neurons (the crista) that runs around the inside of the statocyst approximately in the three main planes of the animal: transverse, longitudinal, and vertical. This crista is subdivided into four segments: the crista transversalis anterior (CTA), the crista longitudinalis (CL), the crista transversalis posterior (CTP), and the crista verticalis (CV) (Budelmann 1977, 1988, 1990). Each crista segment has a sail-like cupula extending into the statocyst lumen, and this is attached to the underlying sensory hair cells via their cilia. The cupulae thus act like swinging doors, moving at right angles to the course of their crista segment and stimulating the underlying sensory hair cells (Budelmann 1990). The four crista segments are innervated by two crista nerves, which enter the statocyst cavity separately through the anterior statocyst wall. The small crista nerve (nervus crista minor) innervates the segment CTA, and the large crista nerve (nervus crista major) the remaining crista segments (CL, CTP, and CV), with a few of its fibers running to CTA segment (Stephens and Young 1982).

In squids, cuttlefishes, and octopuses, each crista segment contains three main cellular elements: the sensory hair cells, which are arranged in up to eight rows; the first-order afferent neurons, which lie close to the sensory hair cells; and the efferent inputs from the brain, which innervate the sensory hair cells and the first-order afferent neurons (Budelmann 1990).

Although the cephalopod crista system shows many parallels with its vertebrate vestibular analogue in its general morphology (Budelmann 1990; Budelmann et al. 1987a; Maddock and Young 1984; Stephens and Young 1982), afferent response characteristics (Williamson and Budelmann 1985), and efferent innervation (Budelmann et al. 1987a; Williamson 1989b), there are major differences. For example, in each crista segment the cephalopod system contains two different types of sensory hair cells: primary sensory hair cells (which have axons that pass toward the brain via the statocyst nerves) and secondary sensory hair cells.
SYNAPTIC INTERACTION BETWEEN HAIR CELLS  

which have no axons passing to the brain) that are in afferent synaptic contact with first-order afferent neurons (Budelmann et al. 1987a; Stephens and Young 1982). In addition, it appears that these two types of sensory hair cells are polarized in opposite directions in cephalopods, such that a stimulus that excites one type of hair cell will inhibit the other type in the same segment and vice versa (Budelmann 1977; Budelmann et al. 1987a; Williamson 1990). In contrast, a vertebrate crista has only secondary sensory hair cells and these are all polarized in the same direction.

The cephalopod crista/cupula system has particular advantages over other systems when examining cell interactions within the peripheral sensory epithelia. First, the crista/cupula system is readily accessible, being located within a relatively soft cranial cartilage instead of a bony skull. More importantly, the first-order afferent neurons have their cell bodies in the periphery within the crista, adjacent to the sensory hair cells; therefore, both the hair cells and first-order afferent neurons can be impaled simultaneously with intracellular microelectrodes, thus allowing direct examination of their connections. This is in contrast to vertebrate systems where only the terminal processes of the first-order afferent neurons extend into the sensory epithelia, making simultaneous recordings from hair cells and first-order afferents nearly impossible.

Thus this paper exploits the unique advantages of the cephalopod statocyst crista system for electrophysiological recordings and describes the morphologies and physiological connections between the sensory hair cells and first-order afferent neurons in the crista epithelium of the statocysts of the squid, Alloteuthis subulata, together with the impact of the efferent innervation on these cell types. A short preliminary report of some of this work has been published (Chrachri and Williamson 1993).

METHODS

The squid A. subulata used in this study were caught locally and maintained in seawater holding tanks untilNeeded. Thirty-two squid (mantle lengths 4.0–9.5 cm) were used. For electrophysiological experiments, an animal was killed by decapitation and the statocysts and surrounding cartilage dissected free. The tissue was then pinned out in a small dish containing cooled, filtered artificial seawater [composition (in mM) 430 NaCl, 10 KCl, 10 CaCl$_2$, 50 MgCl$_2$, and 10 N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid, buffered at pH 7.6], and the right or left statocyst opened carefully to preserve the CTA and its nerve (nervus crista minor) (Stephens and Young 1982). Intracellular recordings then could be obtained from sensory hair cells and/or first-order afferent neurons within the CTA segment. In the present experiments, we report only recordings from sensory hair cells in the outermost dorsal and outermost ventral rows of hair cells in the CTA (Fig. 1A). These were impaled under direct visual control by placing the electrode immediately above the cell and then advancing until the electrode tip touched the outer membrane, as indicated by a small deflection of the voltage trace, and then advancing a few micrometers until a cell resting potential was observed. If a cell resting potential was not obtained within a few microns or a succession of voltage deflections were observed, then the electrode was withdrawn and a new penetration made at another cell. This procedure, in association with those described later, gave a high degree of certainty that we recorded only from the cells specified.

Primary sensory hair cells and small first-order afferent neurons could be identified physiologically during a recording session by electrically stimulating the small crista nerve (nervus crista minor) to evoke an antidromic action potential in the recorded cell. For this purpose, a pair of fine Teflon-coated wires (0.25-mm diam) was inserted into the small crista nerve near the point where the nerve passes through the cartilage on its way to the brain. Such stimulation also activated many of the efferent fibers within the small crista nerve (Stephens and Young 1982).

Intracellular recordings were made with glass microelectrodes

FIG. 1.  
A: diagram of a cross-section through the anterior transverse crista segment (CTA) in the statocyst of the squid Alloteuthis subulata showing the 3 main types of cells studied: primary sensory hair cells (PH, Q), secondary sensory hair cells (SH, ø), and both large first-order afferent neurons (LN) and small first-order afferent neurons (SN, □). Diagram is provisional in that the cells not labeled have not yet been conclusively identified as the types indicated. After Williamson 1989b.  
B–D: fluorescent light micrographs of cells filled with Lucifer yellow.  
B: LN. Note the axon that extends into the small crista nerve.  
C: SH located on the outermost ventral side of the anterior transverse crista segment. Note processes extending from the base of the SH.  
D: PH located on the outermost dorsal side of the anterior transverse crista segment.  
Three photos were taken from 3 different whole-mount preparations. Bars: 20 μm (B), 10 μm (C and D).
fig. 2. lucifer yellow fills of 3 secondary sensory hair cells within the cta segment, well distant from each other, reveal the spatial orientation of the processes that extend from the bases of the hair cells. A: at low magnification, the photomicrograph shows that the processes of the sh on the right go toward the right side of cta segment, whereas those from a sh on the left go toward the left side of cta segment, and those from a sh in the center have only small processes. B: higher magnification of the sh on the left of the cta segment. C: higher magnification of the sh located in the center of the cta segment. D: higher magnification of the sh on the right of the cta segment. Bars: 60 μm (A), 30 μm (B–D).

filled with 3 M potassium chloride or 4 M potassium acetate to give tip resistance of 40–60 MΩ. A conventional bridge circuit was used for both recording and injection of current pulses through a single microelectrode. In some experiments, the sensory hair cells and first-order afferent neurons were stained with the fluorescent dye lucifer yellow (Stewart 1978). In these cases, microelectrodes were filled with 3% lucifer yellow in 3 M lithium chloride. Cells were stained using a pulse protocol of 0.2-s duration, 0.5-nA hyperpolarizing current at 1 Hz for 10–20 min. The preparation was then fixed in 4% paraformaldehyde, dehydrated in alcohol, and cleared in methyl salicylate. Lucifer-filled cells were photographed with an epifluorescence microscope.

results

morphology

primary sensory hair cells. The sensory cells in the outermost hair cell row on the dorsal side of the squid cta are primary sensory hair cells (PHs) that each have a centripetally running axon in the crista nerve (fig. 1, A and D). Intracellular injection of the fluorescent dye lucifer yellow into these cells revealed that the cell bodies are ~10 μm in width. At the base of each PH, a single axon leaves and immediately enters the small crista nerve without branching or giving off processes or axon collaterals (i.e. the axon runs immediately perpendicular to the crista segment, away from the sensory epithelium). No dye coupling between PHs was observed.

secondary sensory hair cells. The sensory cells in the outermost hair cell row on the ventral side of the cta segment of the squid statocyst are secondary sensory hair cells that do not have centripetally running axons (fig. 1A and C) and therefore, unlike the PHs, the secondary sensory hair cells do not show an antidromic spike after stimulation of the small crista nerve. This permits a clear electrophysiological identification between primary and secondary sensory hair cells. injections of the fluorescent dye lucifer yellow into these sensory hair cells have shown that they have processes extending from their bases, which run parallel to the direction of the crista segment (fig. 2) and can extend for ~200 μm along this crista segment. There appears to be a correlation between the position of the secondary sensory hair cells in the cta segment and the direction of the processes, with secondary sensory hair cells on the right of cta segment sending their processes to the right (as viewed from an anterodorsal direction) and those on the left having processes going to the left (fig. 2). Secondary sensory hair cells in the center of this crista segment had only short processes (fig. 2, A and C). No processes were observed to cross

fig. 3. morphology of 2 small first-order afferent neurons within the cta segment. A and B: neurons were stained with lucifer yellow through the recording microelectrodes. They have centripetal axons that run underneath the sensory hair cells in an almost dorsal direction where they give off small processes in close apposition to the secondary sensory hair cells (→). Bars: 10 μm.
from one segment of the crista to a neighboring segment, i.e., from CTA to CL.

**First-order afferent neurons.** The third cell type studied in this report are the first-order afferent neurons. In light and electron microscopical studies of *Octopus vulgaris*, Budelmann et al. (1987a) demonstrated that the first-order afferent neurons can be divided into two types on the basis of their size and location within the crista segment: the first type is the large neurons (LN) with large somata (diameters between 20 and 35 μm). These occur proximally in the epithelium, underneath and between the base of the secondary sensory hair cells, or ventrally to the side (Fig. 1, A and B). The second type is the small neurons (SN) with small somata (diameters between 5 and 15 μm). These always occur ventral to the large neurons, underneath and ventral to the secondary sensory hair cells (Figs. 1A and 3). They do not have a regular arrangement but are scattered and can extend ≈75-μm ventral from the middle of the crista ridge.

Intracellular injection of Lucifer yellow into the small first-order afferent neurons in squid showed that these neurons give off a single axon that passes, without turning, directly underneath and across the sensory hair cells. At the level of the hair cells, however, many of the SNs branch and give off small processes from their axon in close apposition to the secondary sensory hair cells (Fig. 3, A and B).

**Electrical activity and cell coupling**

**Primary sensory hair cells.** For electrophysiological recordings, a PH was impaled with a microelectrode under direct visual control, and its identity confirmed by observing an antidromic spike (Fig. 4B) evoked by electrical stimulation of the small crista nerve. The PHs had a mean membrane resting potential of −61 ± 3 mV (mean ± SD; n = 85). Most of the PHs showed spontaneous electrical activity consisting of large, constant amplitude depolarizations, resembling action potentials, and small depolarizations, resembling post synaptic potentials (PSPs; Fig. 4A). The large spontaneous depolarizations had mean amplitudes of 30 ± 10 mV (n = 55) and, for each hair cell, had the same amplitude and time course as the evoked antidromic action potential. The large depolarizations, therefore, are cell action potentials. These are probably not actively conducted into the hair cell soma but spread decrementally from the axon to the soma recording site. The small depolarizations, or PSPs, often could be divided into small and large types, according to their amplitudes (Fig. 4). As well as occurring spontaneously (Fig. 4A), both types of PSP also could be evoked by electrical stimulation of the small crista nerve (Fig. 4B). With increasing stimulus amplitude, first a constant-latency one-for-one PSP with small amplitude occurred; then a larger amplitude PSP, and finally an antidromic spike (Fig. 4B). Because the antidromic spike and PSPs had the same latency, the PSPs are therefore unlikely to be produced via chemical synapses onto the cells, for example from the many efferent axons present in the small crista nerve because this would result in a 3- to 15-ms delay. It is more likely that the PSPs are due to spikes in neighboring PHs being decrementally conducted into the primary sensory hair cell recorded from, through an electrical synapse, implying that these PHs are electrically coupled.

The nature of this electrical coupling between the PHs was
investigated by making simultaneous intracellular recordings from pairs of closely lying PHs. An analysis of the spontaneous activity recorded from pairs of neighboring PHs revealed that spikes in one PH (PH₂) resulted in a one-for-one transmission to the neighboring PH (PH₁; Fig. 5A). Such electrical transmission can be seen in the neighboring primary hair cell either as a PSP (Fig. 5A) or as a synchronous spike (not shown). Stimulation of the small crista nerve induced an antidromic spike in PH₁, which in turn caused a PSP in the neighboring PH₂ (Fig. 5B).

Injection of depolarizing or hyperpolarizing current pulses into a single PH (PH₂) depolarized or hyperpolarized respectively a neighboring PH (PH₁; Fig. 6, A and B). Figure 6C shows that spike firing frequency in the neighboring PH (PH₁) increased with increasing intensities of the depolarizing current injected into PH₂. The strength of electrical coupling is expressed by the coupling coefficient (k), which is the ratio of the voltage deflection (V₁) in the electrically coupled cell PH₁ to the voltage deflection (V₂) in the primary hair cell into which the current was injected PH₂. In the experiment illustrated in Fig. 6, the coupling coefficient is low, ~0.16. Each time two neighboring PHs were impaled, the type of interaction between them was always found to be electrical coupling. However, the coupling coefficient varied between 0.02 and 0.4 with a mean coefficient of 0.25 ± 0.12 (n = 30). Part of this variation is likely to be due to the technical difficulties in impaling neighboring PHs, for the cells are

FIG. 6. Electrical coupling between 2 PHs within the CTA segment. A: injection of a depolarizing (0.5 nA) and hyperpolarizing (~0.5 nA) current into PH₂ depolarizes and hyperpolarizes PH₁. B: spike frequency is expressed as a function of the amount of the depolarizing current injected into PH₁. Graph shows that the spike frequency of the 2 PHs increases with increasing current injected into PH₂.

FIG. 7. A: intracellular recording from a small first-order afferent neuron within the CTA segment displaying a repetitive discharge of spikes. B: stimulation of the small crista nerve evokes an antidromic spike in the SN (*). C: injection of a depolarizing current into SN₂ depolarizes SN₁. D: injection of a hyperpolarizing current into the same SN₂ hyperpolarizes SN₁.
~10 μm in width and sometimes the coupling coefficient is likely to be measured between primary hair cells separated along the row by one or more PHs, and this will reduce significantly the measured coupling coefficient.

**FIRST-ORDER AFFERENT NEURONS.** Intracellular recordings were obtained from small first-order afferent neurons lying ventral to the crista segment (CTA). These cells had membrane resting potentials around $-47 \pm 3$ mV ($n = 60$), but only 20% showed any spontaneous spiking activity (Fig. 7A). The amplitude of the spikes observed was low, $3.5 \pm 0.55$ mV ($n = 12$), indicating that the spike initiating zone was some distance from the soma recording site and that the spike did not actively invade the soma. The small first-order afferent neurons have their axons in the small crista nerve and therefore produce an antidromic action potential when this nerve is electrically stimulated (Fig. 7B); again the antidromic spike obtained was small.

Simultaneous intracellular recordings from pairs of neighboring small first-order afferent neurons demonstrated that these neurons also are coupled electrically. Injection of depolarizing or hyperpolarizing currents into a small first-order neuron ($SN_2$) depolarized or hyperpolarized, respectively, a neighboring small first-order neuron ($SN_1$) (Fig. 7, C and D). The coupling coefficient between neighboring pairs of small first-order afferent

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**FIG. 8.** Efferent responses in PHs of CTA segment. *A*: electrical stimulation of the small crista nerve induces an antidromic action potential ($\ast$) followed by an excitatory postsynaptic efferent response in a PH in the CTA segment. Sometimes the efferent excitatory postsynaptic potential (EPSP) reaches the threshold for generating spikes in the PH, which results in a spike superimposed on the efferent EPSP ($\rightarrow$). *B*: amplitude of the efferent EPSP increases with increasing stimulus intensity. *C*: sometimes the efferent response consists of an efferent EPSP (○) followed by an efferent inhibitory postsynaptic potential (IPSP, ●). $\ast$, antidromic spike induced by the electrical stimulation.

**FIG. 9.** *A*: stimulation of the small crista nerve induces an efferent EPSP in $SN_1$ of the CTA segment and a mixed efferent response in $SN_2$ consisting of an efferent EPSP (○) followed by an efferent IPSP (●). *B*: in another experiment, stimulation of the small crista nerve induces only an efferent IPSP (●) in $SN$. *C*: amplitude of the efferent EPSP induced in $SN$ increases with increasing stimulus intensity, suggesting a recruitment of new efferent fibers. $\ast$, antidromic spikes induced in $SN$s of the CTA by the stimulation of the small crista nerve.
neurons was between 0.12 and 0.45, with a mean coefficient of 0.29 ± 0.12 (n = 5).

Electrical coupling between large first-order afferent neurons, which lie directly beneath the sensory hair cells, was not tested as these are very difficult to impale in pairs, simultaneously.

**Effect of efferent stimulation**

Cephalopod statocysts receive a large efferent innervation (Budelmann et al. 1987a; Stephens and Young 1982). Stimulation of the small crista nerve activates efferents that run to the CTA segment.

**FIRST-ORDER AFFERENT NEURONS.** The first-order afferent neurons in Octopus also have been shown to receive a large efferent innervation (Budelmann et al. 1987a). Intracellular recordings were made simultaneously from two small first-order afferent neurons of the CTA segment while the small crista nerve was stimulated electrically (Fig. 9A). In SN1, this stimulation induced an efferent EPSP of 7.5 mV in amplitude, whereas in SN2, the same electrical stimulation caused a mixed efferent response, consisting of an efferent EPSP (4.5 mV) followed by an efferent IPSP (1.7 mV). This electrical stimulation also induced an antidromic spike in SN2. Because the two neurons showed different responses to the same stimulus, it is likely that they received a different subset of the efferent innervation. In some cases, the efferent induced response was predominantly inhibitory (Fig. 9B). The amplitude of the efferent response increased with increasing stimulus intensity, as shown in Fig. 9C, probably indicating the recruitment of new efferent fibers and implying that each neuron is innervated by many efferent fibers.

**Chemical versus electrical synapses in primary sensory hair cells**

To investigate the nature of the PSPs induced by spikes in neighboring PHs and EPSPs evoked by stimulating the small crista nerve, the membrane potential of the PH was changed by injection of steady depolarizing and hyperpolarizing currents through the recording electrode in the hair cell in which the PSPs were recorded. Depolarizing or hyperpo-

![Diagram](https://example.com/diagram.png)

*FIG. 10. A: amplitude of the depolarizing PSP recorded in PH2 of the CTA segment remains unchanged whether PH2 is hyperpolarized (---) or depolarized (-----). B–D: by contrast, the amplitude of the efferent EPSP changes when PH is depolarized or hyperpolarized. B: in the control, at a resting membrane potential of −60 mV, stimulation of the small crista nerve induces an antidromic spike (*) followed by an efferent EPSP. C: injection of a steady hyperpolarizing current into PH increases the amplitude of the efferent EPSP. D: injection of a steady depolarizing current into PH decreases the amplitude of the efferent EPSP. *, antidromic spike.*
FIG. 11. Electrical coupling between SH and SN of the CTA segment. A: injection of a depolarizing current into a SH depolarizes a neighboring SN. B: injection of a hyperpolarizing current into SH hyperpolarizes the same neighboring SN. C: injection of a depolarizing current into a SH depolarizes SN and initiates repetitive spikes. SN was injected with a sustained depolarizing current to bring it close to the threshold for spike generation.

larizing PH$_2$ produced no change in the amplitude of the PSP produced by the spontaneous spikes recorded in a neighboring PH$_1$ (Fig. 10A). By contrast, the amplitude of the efferent EPSP, induced by electrical stimulation of the small crista nerve, changed significantly when the postsynaptic PH was injected with either steady depolarizing or hyperpolarizing currents. At a resting membrane potential of -60 mV, stimulation of the small crista nerve induced an antidromic spike followed by an efferent EPSP of ~16 mV (Fig. 10B). Injection of a steady hyperpolarizing current into this primary sensory hair cell increased the amplitude of the efferent EPSP to 22.7 mV (Fig. 10C), whereas injection of a steady depolarizing current into this PH decreased the amplitude of the efferent EPSP to 5.5 mV (Fig. 10D). These data support the view that the PSP from neighboring primary hair cell activity is an electrical effect, whereas the PSP from the efferent nerve stimulation is mediated via chemical synapses.

**Synaptic interaction between primary and secondary sensory hair cells**

Simultaneous intracellular recordings from closely opposed PHs on the outermost dorsal and secondary sensory hair cells on the outermost ventral side of the crista revealed no signs of electrical or chemical synaptic interactions.

**Synaptic interactions between secondary hair cells and small first-order afferent neurons**

From ultrastructural evidence, Budelmann et al. (1987a) argued that the secondary sensory hair cells make afferent synaptic contact with the first-order afferent neurons. We investigated this possibility by performing simultaneous intracellular recordings from a secondary sensory hair cell and a closely lying small first-order afferent neuron. In 70% of our experiments ($n = 45$), injection of a depolarizing current into a secondary sensory hair cell resulted in a small depolarization of the impaled small first-order afferent neuron (Fig. 11A), whereas injection of a hyperpolarizing current into the secondary sensory hair cell hyperpolarized the small first-order afferent neuron (Fig. 11B), implying the existence of electrical coupling between them. This coupling was rectifying, in that it occurred in only one direction from the secondary sensory hair cell to the small first-order afferent neuron. The coupling coefficient between the two types of cells was always very low, between 0.04 and 0.12 with a mean coefficient of $0.08 \pm 0.023$ ($n = 10$). However, as shown in Fig. 11C, if the small first-order afferent neuron was brought close to the spike firing threshold, by the injection of a small positive current, then spiking of this cell could be evoked by depolarizing the secondary sensory hair cells, thus indicating a functional connection between these cells. In the remaining 30% of our experiments, no synaptic interaction between a secondary hair cell and a neighboring small first-order afferent neurons was observed.

**Discussion**

This report describes the morphology, synaptic connectivity, and the efferent responses of sensory hair cells and first-order afferent neurons in the sensory epithelium of the CTA segment of squid statocysts. It is anticipated that the central findings of cell coupling and connectivity also will apply to the cells in the other segments that make up the squid statocyst crista (i.e. CL, CTP, and CV) and also may be applicable to the crista of other cephalopods, such as those of cuttlefishes and octopuses.

**Terminology of primary and secondary sensory hair cells**

Before proceeding, it should be noted that our results have introduced a possible complication into the terminology currently used to describe sensory hair cells. Previous studies on cephalopods have relied on the clear morphological characteristic of the presence or absence of an axon to divide the sensory hair cells into PHs (those with an axon) and secondary sensory hair cells (those without an axon) (e.g.,
Budelmann and Thies 1977; Budelmann et al. 1987a; Klein 1932). However, the dye fills reported here have demonstrated that many of the hair cells in the outermost ventral hair cell row of the CTA segment, previously described in octopus (e.g., Budelmann et al. 1987a) and squid (Williamson 1989b) as secondary sensory cells, have long processes, that may carry action potentials. Thus there is an argument that these cells should be categorized as primary sensory cells. However, these long processes do not pass into the crista nerve and hence do not project directly to the CNS and thus differ from the axons found in the hair cells previously described as primary sensory hair cells. Mindful of this difference, we are reluctant to expand the terminology further and therefore have termed those hair cells that do not have axons in the crista nerve as secondary sensory hair cells. This is in agreement with the terminology used for octopus (cf. Budelmann et al. 1987a) and avoids the complication of having hair cells in the same crista row referred to as primary or secondary sensory cells, depending on whether we can demonstrate by dye injection that they have a long process or not.

**Morphology**

Dye fills of cells within the epithelium of the CTA segment have confirmed that the hair cells in the outermost dorsal row are PHs that have a simple morphology, consisting of a cell soma within the crista ridge and an axon passing dorsally into the crista nerve towards the CNS, with no branching or processes apparent within the sensory epithelia. The small first-order afferent neurons on the ventral side of the CTA segment are more complex, for these have processes emanating from the axon as it passes from the ventrally lying cell body beneath the hair cell area; this is presumably the dendritic area where the secondary sensory hair cells make contacts with the afferent neurons. From the length of the dendrites, it seems likely that direct contact will be made with only a small number of hair cells within a fairly localized area (cf. Budelmann et al. 1987a). Dye fills of cells in the outermost ventral row of hair cells in the CTA segment have shown that these are secondary sensory hair cells, which do not have axons passing into the small crista nerve, but which can have long processes extending along the segment. Such long processes have not been previously described for the sensory hair cells of other cephalopod statocysts. Klein (1932), however, described the crista hair cells in the outermost ventral row of hair cells in the cuttlefish statocyst (his "distalen Nebenzellen") as having an axon, and it may be that these are not PHs but secondary sensory hair cells that also have long processes.

The extensive branching patterns of these axon-like processes in the squid statocyst crista may complicate our view of the connections of the secondary sensory hair cells to the primary afferent neurons for these processes may make extensive connections with cells along the crista in a way that divides the crista into separate zones. Secondary hair cells in the center have only a few short processes, whereas those to the right and left have long processes extending respectively to the right and left along the crista. From the position of these processes in the epithelium, they could make contact with other hair cells or afferent neurons. These long processes have not been identified in other invertebrates or vertebrate secondary sensory hair cells, although some short processes, =8 μm in length, have been reported in secondary sensory hair cells of the octopus macula and crista (Budelmann and Thies 1977; Budelmann et al. 1987a; Colmers 1977). The function of these long processes is not yet known, but it is possible that they are responsible for electrical coupling between secondary hair cells along the entire row of secondary hair cells, for it has been found that electrical coupling along the row is not abolished when a single hair cell in the row is damaged (Williamson 1989a). Another role for these long processes of the secondary sensory hair cell is probably to carry action potentials, and this is supported by both conventional microelectrode recordings (unpublished observation) and patch-clamp experiments indicating that many of these hair cells express tetrodotoxin-sensitive, voltage-dependent sodium currents and that these currents are associated with the secondary sensory hair cell processes (Williamson 1995). Some vertebrate secondary sensory hair cells also carry action potentials, for example, those in the cochlea of alligator (Evans and Fuchs 1987) and the sacculus of goldfish (Sugihara and Furukawa 1989), and it has been proposed that this may provide a time locked afferent output in response to low-frequency stimuli and that this could enhance time-dependent operations such as sound localization.

**Electrical coupling and interconnections**

The secondary sensory hair cells located on the outermost ventral side of squid CTA segment are coupled electrically along the length of a crista segment, with a coupling coefficient of =0.6 (Williamson 1989a). We now have demonstrated that small first-order afferent neurons also are coupled electrically to each other and confirmed an earlier report (Chrachri and Williamson 1993) that the PHs, located on the outermost dorsal side of the CTA segment, also are coupled electrically, but this time, with a very variable coupling coefficient (between 0.02 and 0.4). The function of electrical coupling between the small first-order afferent neurons again may be to increase the sensitivity of the system by providing a better signal-to-noise ratio, at the expense of frequency response (cf. Williamson 1989a), or perhaps to synchronize the activity of the small first-order neurons and thus provide a time locked output in response to rapid signals such as vibrations.

Electrical coupling between neurons within relatively large populations of cells has been described in a variety of vertebrate and invertebrate preparations (Bennett 1966; Levitan et al. 1970). It is widely accepted that electrical coupling plays an important role not only in synchronizing the firing of pools of neurons with similar function (Bennett et al. 1967; Chrachri and Clarac 1989), but also in the speed of conduction needed in fast reaction such as an escape system (Auerbach and Bennett 1969; Zucker 1972).

Although electrically coupled cells often show some dye coupling, we have not seen any dye spreading between hair cells in the statocyst of squid even when using small molecular weight fluorescent dyes, such as Lucifer yellow and 6-carboxyfluorescence, which are reported to pass more easily between coupled cells (Santos-Sacchi 1986; Stewart 1978).
However, there have been a number of reports, particularly in mollusks, of cells that are electrically coupled but do not show any dye coupling (e.g., Ewadinger et al. 1994).

There is no electrical coupling between the outermost ventral secondary sensory hair cells and outermost dorsal primary sensory hair cells in the squid statocyst CTA segment. This is hardly surprising as these hair cells are polarized in opposing directions (Budelmann 1977; Budelmann et al. 1987a; Williamson 1990) and, therefore, such coupling would act to cancel out the mechanosensory responses of these cells and result in a loss of directional sensitivity.

Simultaneous intracellular recordings from a secondary sensory hair cell and a closely lying small first-order afferent neuron demonstrated electrical coupling between them. This was an unexpected finding for there is good morphological (Budelmann et al. 1987a) and physiological evidence (Tu and Budelmann 1994) from other cephalopod species that there is a chemical synapse between these cell types. Dye injection into small first-order afferent neurons revealed ramifications close to the location of the secondary sensory hair cells. The role of these ramifications is not yet clear, however, one can speculate that the electrical coupling (this report) as well as chemical synapses (Budelmann et al. 1987a; Stephens and Young 1982) between secondary sensory hair cells and small first-order afferent neurons may take place close to these ramifications. This electrical coupling, where it is present, is in one direction only, from secondary sensory hair cells to small first-order afferent neurons. Unlike the PHs, which have a direct connection (via their axons) to the CNS, the secondary sensory hair cells are connected indirectly to the CNS via either electrical synapses (this report) or via chemical synapses (Budelmann et al. 1987a; Stephens and Young 1982).

**Efferent response**

The experiments described in this report have shown that the primary and secondary sensory hair cells, as well as the first-order afferent neurons, of the squid statocyst crista receive both excitatory and inhibitory efferent inputs (Budelmann et al. 1987a; Williamson 1989b). The PHs seem to receive fewer efferent inputs than the secondary sensory hair cells and the small first-order afferent neurons. Three tests were used to characterize the nature and properties of both the PSPs caused in PHs by spikes from neighboring PHs and the EPSPs induced by efferent stimulation. First, the latency of the efferent responses is much longer (3–15 ms) compared with the latency (1.1 ms) for the PSPs seen in the PHs, which is similar to that observed for the antidromic spikes recorded from the PHs and the small first-order afferent neurons. Second, changes in the membrane potentials of both hair cells and first-order afferent neurons through either a sustained depolarization or hyperpolarization was capable of changing the amplitude of the efferent responses but not the amplitude of the PSPs recorded in the primary sensory hair cells. Third, the excitatory efferent responses are blocked in the presence of a solution of 1 mM cobalt chloride, whereas the PSPs are not (Chrachri and Williamson 1993). These three tests imply that the efferent responses are mediated via chemical synapses, whereas the PSPs recorded in primary sensory hair cells are mediated through electrical synapses between neighboring PHs.

Excitatory and/or inhibitory efferent responses were recorded in both primary and secondary sensory hair cells as well as in small first-order afferent neurons. Two separate hypotheses can be proposed to explain this apparent dual efferent response. One hypothesis is that there is only one type of synaptic input and that differences in the cell membrane potential, around a reversal potential for the efferent synaptic input, caused it to appear sometimes depolarizing and sometimes hyperpolarizing. This is unlikely to be the case here, however, because the two types of efferent inputs had different latencies, with the excitatory response always preceding the inhibitory response, they often were seen simultaneously in the same cell, and they previously have been shown to have different reversal potentials in secondary sensory hair cells (Williamson 1989b). The second hypothesis is that these excitatory and inhibitory efferent responses are due to two separate populations of efferent fibers, one excitatory and the other inhibitory; these two populations could have different conduction velocities, perhaps with different axon diameters or different kinetics for their synaptic transmission. No bimodal distribution of efferent diameters was found in octopus (Budelmann et al. 1987a), but there is morphological and histochemical evidence that there are two different efferent transmitters (Auerbach and Budelmann 1988; Budelmann et al. 1987a; Stephens and Young 1982) between secondary sensory hair cells and the small first-order afferent neurons. Two separate transmitters (ACh and catecholamines) have an excitatory effect on the crista afferent activity (Williamson 1989b). The functional role of these efferent inputs is not yet clear, but recent investigations have shown that ACh and catecholamines affect the strength of electrical coupling between sensory hair cells by changing sensory hair cell input resistances and time constants (Williamson and Chrachri 1994; unpublished data). Similar results have been reported for dopamine in the control of electrical coupling in the vertebrate retina (Teranishi et al. 1983).

The dual efferent input to the statocyst provides the animals with a very sophisticated control system for adjusting the level of the afferent activity, for it could depress or enhance the input from individual cell types. For example, the very large, predictable afferent activity that results from a jet propelled escape response could be minimized through the inhibitory system, whereas the afferent gain of the system could be increased via the excitatory efferent system during fine maneuvering movements before prey capture.

**Comparisons with other animals**

The main differences between vertebrate and invertebrate balance organs are in the type of hair cells and their interconnections. Most invertebrates have only PHs (reviewed by Budelmann 1988), whereas vertebrates have secondary sensory hair cells; it is only the coleoid cephalopods that have both types of hair cell in the same sense organ. Electrical coupling also occurs between sensory hair cells in the statocyst of simpler mollusks, such as gastropods (Dewiler and Alkon 1973), as well as in cephalopods, but electrical coupling is believed to be rare in vertebrate hair cell systems, although there is some evidence of gap junctions (Miller and Beck 1990).

Although vertebrate and cephalopod statocysts have simi-
lar afferent response characteristics (Williamson and Budelmann 1985) and efferent systems (Williamson 1989b) and are part of similar ocular reflexes (Budelmann and Young 1984), it remains to be seen whether there are common features to the central processing systems of the very different brains of these two groups.

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