

## **DIRECTIONAL SENSITIVITY OF HAIR CELL AFFERENTS IN THE *OCTOPUS* STATOCYST**

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### **Summary**

Changes in threshold sensitivity of hair cell afferents of the macula and crista of the *Octopus* statocyst were analyzed when the hair cells were stimulated with sinusoidal water movements from different directions. The experiments indicate that cephalopod statocyst hair cells are directionally sensitive in a way that is similar to the responses of the hair cells of the vertebrate vestibular and lateral line systems, with the amplitude of the response changing according to the cosine of the angle by which the direction of the stimulus (the deflection of the ciliary bundle) deviates from the direction of the hair cell's morphological polarization.

### **Introduction**

Both vertebrates and invertebrates have developed sophisticated sense organs containing mechanoreceptive hair cells. In vertebrates, the morphology and physiology of these cells have been extensively studied, with recent work focusing on the transduction process of the hair cells of the vestibular and lateral line systems (for reviews, see Flock, 1971; Platt, 1984; Howard *et al.* 1988; Roberts *et al.* 1988; Hudspeth, 1989; Jakobs and Hudspeth, 1990; Ashmore, 1991; Corwin and Warchol, 1991; Pickles *et al.* 1991; Pickles and Corey, 1992).

A well-known characteristic of these hair cells is their morphological and physiological polarization. Morphologically the hair cell's polarization is defined by any of the following criteria: (1) the eccentric position of the kinocilium relative to the bundle of stereovilli (=stereocilia), (2) the orientation of the internal  $9 \times 2 + 2$  tubuli content of the kinocilium, (3) the orientation of the basal foot structure on the basal body of the kinocilium, (4) the gradation in height of the stereovilli, or (5) the orientation of the tip links between the stereovilli (e.g. Lowenstein and Wersäll, 1959; Wersäll and Bagger-Sjöbäck, 1974; Wiederhold, 1976; Pickles *et al.* 1991). The hair cell's morphological polarization is of great importance for the interpretation of physiological experiments

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and, therefore, the morphological polarization patterns of hair cell epithelia have been described in detail for many vertebrate species, including humans (for references, see Budelmann, 1979; Platt, 1984). The hair cell's morphological polarization has a clear physiological correlate. Deflection (shear) of the ciliary bundle in the direction of the hair cell's morphological polarization results in a maximal depolarization of the hair cell, whereas deflection in the opposite direction results in a maximal hyperpolarization, with the response amplitude changing as a function of the cosine of the angle by which the direction of deflection of the ciliary bundle deviates from the hair cell's axis of polarization (Lowenstein and Wersäll, 1959; Trinker, 1962; Flock, 1965; Giesen and Klinke, 1969; Hudspeth and Corey, 1977; Shotwell *et al.* 1981; Russell and Richardson, 1987; Ohmori, 1989; Crawford *et al.* 1989; Rüscher and Thurm, 1989).

In invertebrates, mechanoreceptive hair cells are best known in equilibrium and hydrodynamic receptor systems (for reviews, see Budelmann, 1988, 1989). They differ from vertebrate hair cells in that they carry, species specifically, between 1 and 700 kinocilia per cell but – with one exception (coelenterates; Horridge, 1969; Singla, 1975) – no stereovilli. According to their morphology, the invertebrate hair cells can be divided into polarized and non-polarized hair cells. Polarized hair cells are common in cephalopods (Barber, 1968; Budelmann, 1977, 1979; Budelmann *et al.* 1987) but they are rare in other invertebrates (for references, see Budelmann, 1988).

The polarized hair cell of the cephalopod statocyst carries up to 200 kinocilia. These are arranged in an elongated ciliary group that is inclined towards the surface of the cells. All kinocilia of one cell are morphologically polarized in the same direction, which is at right angles to the long axis of the ciliary group and opposite to the acute angle of inclination of the group (Budelmann, 1979). By analogy with the results from the vertebrate hair cells, it has been suggested that, in cephalopods also, the hair cells are directionally sensitive in such a way that deflection of the kinociliary group in the direction of the hair cell's morphological polarization causes a maximal depolarization of the hair cell, and deflection in the opposite direction causes a maximal hyperpolarization (Barber, 1968; Budelmann, 1970). However, this has not yet been shown experimentally. The results of electrophysiological and behavioural experiments, however, have been interpreted in terms of, and fully support, this idea (Budelmann, 1970; Williamson and Budelmann, 1985; Williamson, 1990, 1991).

Stimulating the hair cells of the *Octopus* statocyst receptor systems (macula and crista) with sinusoidal water movements from different directions, and subsequent threshold analyses, indicates that the statocyst hair cells of cephalopods are directionally sensitive in a way that is similar to the responses of the hair cells of the vertebrate vestibular and lateral line systems.

### Materials and methods

Experiments were performed on isolated macula/statolith and crista/cupula preparations of eight statocysts (left and right) of five *Octopus bimaculoides* (Pickford and McConnaughey) (mass range 120–160 g; males and females), caught in the Pacific Ocean near Los Angeles, CA.

### Preparation

The animals were killed by decapitation without prior anaesthesia and the two sphere-like statocyst sacs were carefully removed under sea water from their cartilaginous cavities. One statocyst sac (still unopened) was then transferred in a large-diameter seawater-filled pipette to a small seawater tank containing a rotation platform (see below). The statocyst was placed on the top of a wax cone (diameter of cone platform 2 mm) on top of an acrylic rod in the centre of the rotation platform (Fig. 1A–C). The statocyst sac was then carefully opened with a pair of very fine Vannas scissors opposite to the macula and the first two crista segments ( $C_1$  and  $C_2$ ; see Budelmann *et al.* 1987); from there several radial cuts were made into the statocyst sac. The macula and crista segment  $C_1$  were then positioned in the middle of the wax cone, with the cartilaginous macula plate fitting into a matching indentation in the wax cone, and the statocyst sac tissue segments were folded back and firmly fixed with short stainless-steel insect pins (0.1 mm diameter) to the lateral wall of that cone, thus freely exposing the macula/statolith and crista/cupula segment  $C_1$  to sinusoidal water movements (Fig. 1C). During these procedures, the tissue was handled with extreme care to avoid damaging the contact between the statolith and cupula and their sensory epithelia.

### Rotation device

The rotation platform (Fig. 1A,B) (diameter 105 mm) was in the middle of a small vibration-isolated (NRC Pneumatic Isolation Table, Newport, Fountain valley, CA) acrylic tank (150 mm × 110 mm × 50 mm) filled with sea water (19–22 °C). The platform could be smoothly moved around a central pivot by hand using the attached handle. A small micromanipulator firmly attached to the same platform carried the recording electrode and was counterbalanced by a lead weight on the opposite side of the platform. To change the direction of the stimulus relative to the sensory epithelia (see below), the platform was very slowly rotated in steps of 20°. The placement of the micromanipulator stand and handle limited the maximum rotation of the platform to 290°. After each movement of the platform to a new position, the preparation was allowed a 1 min recovery period before any water movement stimuli were applied.

### Stimulation

Mechanical stimulation of the hair cells of the macula/statolith and crista/cupula systems with sinusoidal water movements was produced by a vibrating sphere (14.6 mm in diameter), fixed to a titanium rod (diameter 3 mm) and driven in the axis of the rod by a vibrator (model 102, Ling Dynamic Systems, Royston, England) mounted outside the tank. The rod passed through a watertight high-density foam rubber diaphragm (diameter 12 mm) in the lateral wall of the tank (Fig. 1A,B). The axis of the rod was horizontal and 3 mm above the macula and crista epithelia. The ball surface was 9–30 mm from the sensory epithelia and 7–10 mm from the water surface. The sensory epithelia were 20 mm from the floor of the rotation platform and 15 mm from the water surface. The vibrator was driven by a function generator (Exact Electronics, Tillamook, OR). Stimulation frequencies were 50, 100 and 200 Hz, although 100 Hz was used more often because it

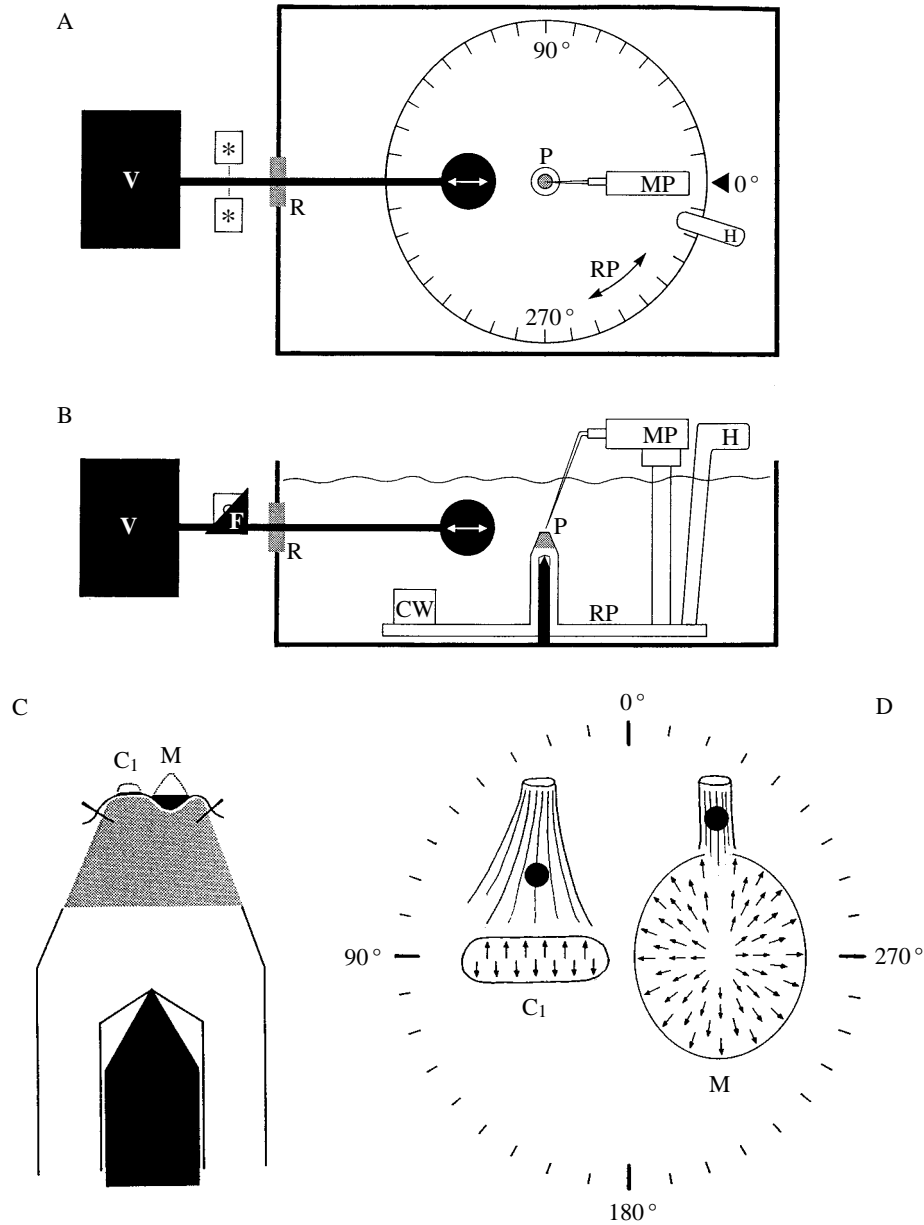


Fig. 1

was the most effective stimulus. Sphere displacement was monitored with a photodiode such that the light beam going to the diode was partially blocked by a metal flag attached to the rod of the vibrating sphere. For the frequencies and amplitudes used, movements of the sphere caused sinusoidal changes in the photodiode voltage output. See Kalmijn (1988), Coombs *et al.* (1989) and Bleckmann *et al.* (1991) for the method of calibration of the peak-to-peak displacement (in  $\mu\text{m}$ ) of the sphere and further details of the sphere and

Fig. 1. Rotation device, seen from above (A) and from the side (B). The preparation (P) is positioned in the middle of a wax cone (stippled; details in C) on top of a rod in the centre of a rotation platform (RP, diameter 105 mm). A micromanipulator (MP) holding the recording electrode is firmly fixed to the platform, as is a handle (H) by which the platform can be smoothly rotated in either direction around a central pivot. Note that in this figure the 360° scale on the platform is put arbitrarily with regard to the coordinates of the platform; during the experiments, the scale was matched to the coordinates (360° scale) of the macula and crista segment, as shown in D. Controlled sinusoidal water movements are produced by a vibrating sphere. Sphere displacement (white arrows) is monitored by a photodiode (\*), the light beam of which is partially interrupted by a metal flag (F) attached to the rod that moves the sphere. CW, counterweight; R, foam rubber diaphragm; V, vibrator. The apparatus allows one to change the direction of the stimulus (sinusoidal water movement) relative to the preparation (hair cells) without changing the stimulus intensity (i.e. the distance between the preparation and the vibrating sphere). (C) Wax cone (stippled) and preparation on top of the rod in the centre of the rotation platform. The statocyst tissue is firmly fixed to the wax cone with short insect pins such that the macula/statolith (M) and crista/cupula (C<sub>1</sub>) systems are exposed to the water movement stimuli; the cupula and cone-like statolith are outlined by dots. (D) Diagram to illustrate the directions of morphological polarization of the hair cells in the macula (M) and first crista segment (C<sub>1</sub>). The 360° scale indicates the directions of the stimulus/sensitivity described in the text and figures. The filled circles on the macula and anterior crista nerves show the position of the recording electrodes. The pattern of morphological polarization is based on data from Barber (1968), Budelmann (1976, 1977) and Budelmann *et al.* (1987).

water movements. The peak-to-peak water displacements caused by the vibrating sphere at the level of the hair cells were calculated according to the method of Harris and van Bergeijk (1962).

#### Recording

Action potentials were recorded extracellularly with seawater-filled polyethylene suction electrodes (inner tip diameter 150–200 µm) from afferent fibres of the macula and anterior crista nerves. Unfortunately, recordings from the secondary sensory hair cells directly, instead of from their neurones, are extremely difficult to make without damage to, or changes to, the crista/cupula and macula/statolith mechanics (see *Morphological background* in Results for details of the cellular organization of the receptor epithelia and their afferent fibres). Recording sites were as shown in Fig. 1D. The electrode was placed onto the nerve fibres using minimal pressure and very little suction. The potentials were amplified with a conventional preamplifier (DAM 80, World Precision Instruments, Sarasota, FL) and, together with the stimulus monitor signal (photodiode voltage output of the sphere displacement), displayed on a cathode ray oscilloscope and, with an additional voice commentary, recorded on a Hewlett Packard FM tape recorder. The isolated preparation remained viable, and the recordings stable, for at least 3 h.

#### Threshold

To determine the threshold of the hair cell afferent units for a given stimulus frequency, the amplitude (but not the frequency) of sphere vibration, and thus the peak-to-peak sinusoidal water movement, was systematically increased from below threshold until a clearly identifiable large single unit (which was not necessarily the most sensitive unit;

see also Discussion) could be seen responding in a phase-locked 1:1 manner with the sinusoidal water movement; at this point, the photodiode voltage output was measured for later calculation of the amplitude of the water movement at the level of the hair cells.

#### *Directional sensitivity*

To determine the directional sensitivity of a hair cell afferent unit, the position of the preparation, and thus of the hair cells, relative to the stimulus (i.e. the stimulus direction) was systematically changed by carefully rotating the platform in steps of 20° through a total of 280°. The threshold of the recorded unit was determined at each position. The least effective stimulus direction, however, was difficult to determine precisely (within the 20° interval), because the stimulus amplitude necessary to elicit a threshold reaction at these directions increased to a level that caused stimulus and water movement artefacts.

#### *Graphical presentation of data*

The data are presented as graphs of the stimulus amplitude necessary to evoke a threshold response of a single macula or crista afferent unit plotted against the angle between the stimulus direction and the axes of the macula and crista epithelia. The response threshold was calculated in terms of the peak-to-peak water displacement (in  $\mu\text{m}$ ) at the level of the macula and crista hair cells. It has already been shown for vertebrate hair cells (see Introduction) that the directional response of a hair cell can be described as  $A = C \cos \alpha$ , where  $A$  is the magnitude of the hair cell response,  $\alpha$  is the angle between the direction of the applied stimulus and the direction of the polarization of the hair cell, and  $C$  is a constant related to the amplitude of the applied stimulus; the stimulus amplitude is kept constant. In order to demonstrate that a similar relationship is present in the *Octopus* macula and crista hair cells, we have fitted a cosine curve to the threshold data using Sigmaplot (Jandel Scientific, Corte Madera, CA). Note that in our experiments the response amplitude has been kept constant and it was the stimulus amplitude and stimulus direction that were varied. Therefore, we expected to find an inverse cosine relationship, i.e.  $B = K / \cos \alpha$ , where  $B$  is the stimulus amplitude,  $\alpha$  is the angle between the stimulus direction and the axes of the macula and crista epithelia, and  $K$  is a constant related to the magnitude of the response. Note that the steepness of the curve within the given range will change with the value of  $K$  (see Fig. 4).

### **Results**

Stimulation of the crista/cupula and macula/statolith systems of the opened statocyst sac with sinusoidal water movements elicited responses of hair cell afferent units in a phase-locked 1:1 manner with the water movement. Each unit responded at only one phase position within the stimulus cycle. For details of the afferent fibre responses of the *Octopus* statocyst, see Budelmann and Wolff (1973), Williamson and Budelmann (1985) and Williamson (1985, 1988). In all experiments, the sensitivity (threshold) of the units varied in a similar manner when the direction of the stimulus was changed relative to the axis of the macula and crista epithelia. The lowest thresholds were found to be 0.12  $\mu\text{m}$

(crista) and  $0.4\ \mu\text{m}$  (macula) peak-to-peak water displacement (calculated at the level of the hair cells) at 100 Hz (but see Discussion for maximal sensitivity of hair cells).

*Control for biological versus artefactual origin of the response*

Although all experiments were carried out at low (threshold) stimulus intensities, which in itself reduce the likelihood of mechanical artefacts, water movements may have directly affected the recordings. Therefore, the following control experiment was performed with a macula/statolith preparation. First, a clearly identifiable large single unit was selected and its direction of sensitivity and lowest threshold were determined. The statolith overlying the macula was then removed without taking the suction electrode from the macula nerve, i.e. without changing the recording conditions, by first piercing the mucus membrane around the edge of the statolith with the tapered end of a 0.1 mm insect pin and then very carefully lifting off the statolith with fine forceps. This procedure left thick layers of mucus with matching (positive/negative) irregular surfaces on the macula and the ventral side of the statolith. With the statolith removed, no responses of hair cell afferent units could be obtained, even when the macula surface was stimulated with very large sinusoidal water movements. This strongly indicates that the responses obtained in the experiments were not mechanical artefacts but biological events caused by the water movements. In a final step, the statolith was replaced onto the macula in, or as close as possible to, its original position by fitting together the matching positive/negative irregular mucus layers of the statolith and the macula. Renewed stimulation of the macula/statolith system again resulted in a response of the original hair cell afferent unit, although at a slightly higher stimulus threshold compared with that of the first recording (probably because of a change in the mechanics of the 'mucus-coupling' between the statolith and the macula epithelium and hair cells).

*Results from crista hair cells*

*Morphological background*

Data on the gross morphology and fine structure of the crista/cupula system of the *Octopus* statocyst that are relevant for this work are given in Young (1960), Budelmann (1977) and Budelmann *et al.* (1987). The crista is subdivided into nine elongated crista segments ( $C_1$ – $C_9$ ). Each segment carries either a small or a large cupula, which protrudes freely into the cyst cavity and acts like a swinging door, with movements at right angles to the elongated crista segment. Each crista segment is composed of rows of primary sensory hair cells, as well as rows of large and small secondary sensory hair cells which are in synaptic contact with large and small, respectively, first-order afferent neurones. All the hair cells are morphologically polarized at right angles to the elongated crista segments, i.e. in the horizontally arranged crista segments  $C_1$ – $C_6$ , the primary sensory hair cells are polarized dorsally and the secondary sensory hair cells are all polarized ventrally (Fig. 1D). In the present crista experiments, all recordings are from crista segment  $C_1$  (with a small cupula). In *Octopus vulgaris*, crista segment  $C_1$  has an average of 120 dorsally polarized primary sensory hair cells, and 50 small and 60 large ventrally polarized secondary sensory hair cells. Almost certainly, all recordings represent

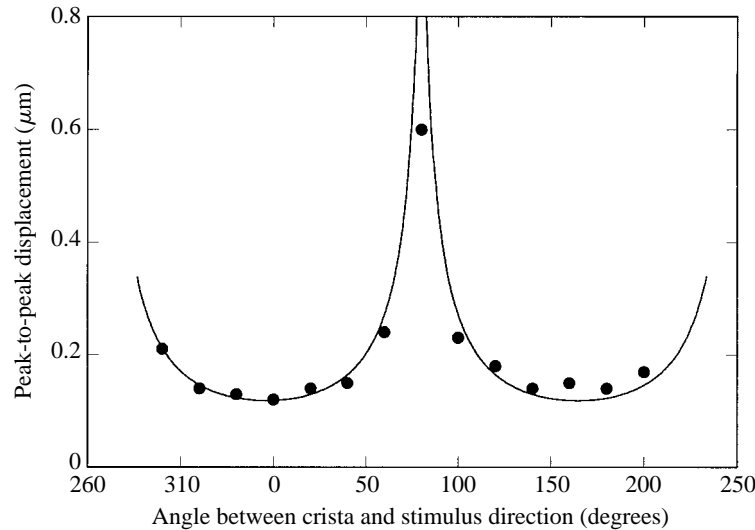


Fig. 2. Directional sensitivity of a single afferent crista unit. The peak-to-peak water displacements (100 Hz) at the level of the preparation that were necessary to elicit a threshold response of that unit (ordinate) are plotted against different directions of the stimulus relative to the crista segment, i.e. relative to the morphological polarization of the unit's corresponding hair cells (abscissa). The curve shows the best-fitted inverse cosine function. Note that, in this and the following figures, the least effective stimulus direction was difficult to determine precisely (within the  $20^\circ$  intervals), because the amplitude of water movement that was necessary to elicit a threshold reaction in that direction increased to a level that caused stimulus and water movement artefacts.

responses of the large first-order afferent neurones of the large ventrally polarized secondary sensory hair cells (see also Williamson and Budelmann, 1985). It is unlikely that the recordings were from axons of the primary sensory hair cells because their extracellularly recorded potentials are generally much smaller and are therefore difficult to analyse (Williamson and Budelmann, 1985).

#### *Physiological results*

In all three crista experiments, stimulation of the  $C_1$  crista/cupula segment with sinusoidal water movements elicited identical direction-sensitive responses of hair cell afferent units. Each unit responded through an angle of  $180^\circ$ , with the direction of highest sensitivity being identical for all the crista units investigated. Fig. 2 shows the peak-to-peak water displacements (at 100 Hz) that were necessary to elicit the threshold responses of a single hair cell afferent unit at different stimulus directions relative to the crista segment and thus relative to the hair cells' direction of morphological polarization. Highest sensitivity (lowest threshold) of the unit occurred when the hair cells were stimulated from a direction parallel to the direction of their morphological polarization; this was at approximately  $0^\circ$  and  $180^\circ$ , which are at right angles to the elongated crista segment (compare Fig. 1D). The sensitivity of the unit decreased (threshold increased) as



a cosine function when the stimulus direction was moved away in either direction from the hair cells' direction of polarization. Lowest sensitivity (highest threshold) occurred when the hair cells were stimulated from a direction at right angles to their morphological polarization; this was at approximately  $90^\circ$ , which is parallel to the elongated crista segment (compare Fig. 1D).

#### *Results from macula hair cells*

##### *Morphological background*

Data on the gross morphology and fine structure of the macula/statolith system of the *Octopus* statocyst that are relevant for this work are given in Young (1960), Barber (1966), Budelman *et al.* (1973), Budelmann (1976), Budelmann and Thies (1977) and Colmers (1977, 1981). The macula is an oval plate of cells that carries a calcareous cone-like statolith. In *Octopus vulgaris*, the macula is composed of about 5000 hair cells, which are all secondary sensory cells. All the hair cells are arranged in concentric rings and are polarized radially outwards towards the macula periphery (Fig. 1D). They synapse onto first-order afferent neurones. A minimum of 2–3 hair cells (but probably up to 50 hair cells) with similar directions of polarization converge onto one afferent neurone as its axon passes underneath the hair cells to join the macula nerve. In the present experiments, all recordings are from such afferent neurones.

##### *Physiological results*

In all eleven macula experiments, stimulation of the macula/statolith system with sinusoidal water movements elicited direction-sensitive responses of hair cell afferent units. Fig. 3 shows the peak-to-peak water displacements (at 100 Hz) that were necessary to elicit the threshold responses of a hair cell afferent unit at different stimulus directions relative to the macula coordinates and thus relative to the hair cells' direction of morphological polarization (compare Fig. 1D). Highest sensitivity (lowest threshold) occurred at approximately  $260^\circ$ . The sensitivity of the unit decreased (threshold increased) as a cosine function when the stimulus direction was moved away in either direction from this position. It reached lowest sensitivity (highest threshold) at approximately  $170^\circ$  and  $350^\circ$ , respectively. All eleven macula units responded through an angle of approximately  $180^\circ$ . As to be expected, however, the stimulus directions for the highest sensitivities (lowest thresholds), determined by curve fittings, varied with respect to the macula coordinates (compare Fig. 1D) between  $170^\circ$  and  $300^\circ$  ( $170^\circ$ ,  $180^\circ$ ,  $200^\circ$ ,  $210^\circ$ ,  $210^\circ$ ,  $250^\circ$ ,  $250^\circ$ ,  $260^\circ$ ,  $260^\circ$ ,  $290^\circ$  and  $300^\circ$ ). Fig. 4 shows the directional sensitivities of two macula hair cell afferent units from the same preparation that had their highest sensitivities at different directions ( $210^\circ$  and  $300^\circ$ ).

#### **Discussion**

The present recordings from octopus crista and macula hair cells/afferent units show, for the first time, the physiological polarization (directional sensitivity) of cephalopod statocyst hair cells that had been postulated on the basis of morphological criteria and by analogy with vertebrate vestibular hair cells (e.g. Barber 1966; Budelmann, 1979). As for

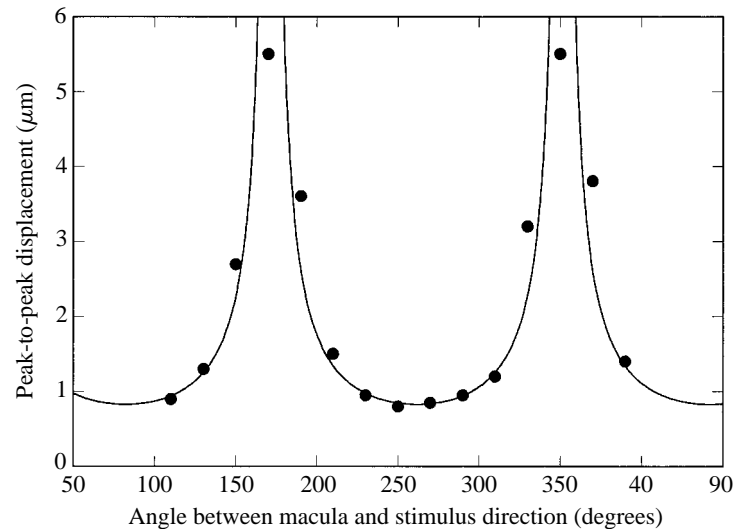


Fig. 3. Directional sensitivity of a single afferent macula unit. The peak-to-peak water displacements (100 Hz) at the level of the preparation that were necessary to elicit a threshold response of that unit (ordinate) are plotted against different directions of the stimulus relative to the macula, i.e. relative to the morphological polarization of the unit's corresponding hair cells (abscissa). The curve shows the best-fitted inverse cosine function.

vertebrate hair cell (for references, see Introduction), the amplitude of the response depends on the cosine of the angle by which the direction of the hair cell's ciliary deflection deviates from the direction of its morphological polarization. Because of the experimental design, the present experiments could not show the sine-like correlation between the amplitude of the stimulus and the strength of the response that is known for vertebrate vestibular hair cells (e.g. Hudspeth and Corey, 1977; Rüscher and Thurm, 1989). This correlation, however, has already been shown for the cephalopod crista/cupula system by measurements of the hair cell's membrane potential in response to cupula displacements of different amplitudes (Williamson, 1990). There is an asymmetry in the amplitude of the response (increase *versus* decrease of the hair cell's membrane potential) to a given cupula displacement in opposite directions, with the amplitude of the response being larger when the cupula, and thus the cilia of a hair cell, is displaced in the direction of the cell's morphological polarization (Williamson, 1990). Together with the present experiments, this clearly shows that cephalopod statocyst hair cells have response characteristics that are similar to those of the vertebrate vestibular and lateral line hair cells (for references, see Introduction).

It is necessary to reiterate that the recordings were not made directly from the hair cells but from their first-order afferent neurones (see *Morphological background* in Results). Nevertheless, with regard to the directional sensitivity, the responses of the neurones can be considered to represent those of a single hair cell, or of several hair cells that coincide in their direction of morphological polarization. This is true for both the crista and the macula neurones, because there is strong morphological evidence that, in the two

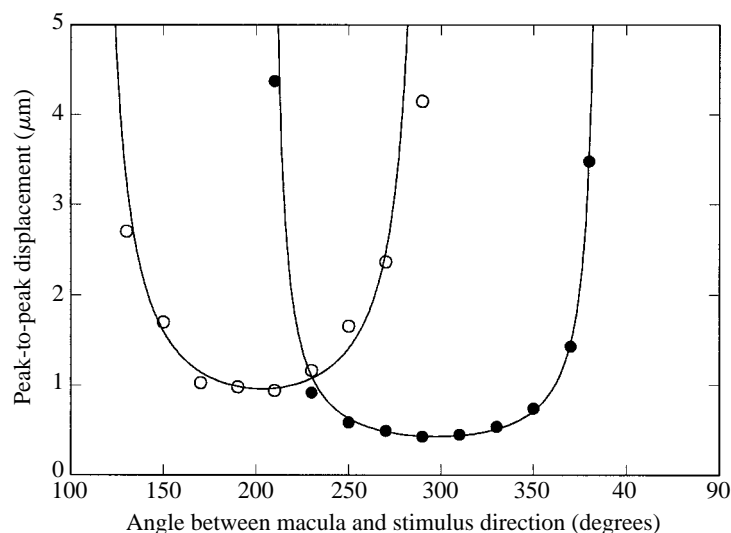


Fig. 4. Directional sensitivities of two afferent macula units (open and filled circles) in the same preparation but with different directions of highest sensitivity ( $210^\circ$  and  $300^\circ$ , respectively). The peak-to-peak water displacements (100 Hz) at the level of the preparation that were necessary to elicit the threshold responses of these units (ordinate) are plotted against different directions of the stimulus relative to the macula, i.e. relative to the morphological polarization of the unit's corresponding hair cells (abscissa). The curves show the best-fitted inverse cosine functions. Note that the right-hand unit has a higher sensitivity (lower minimum) and a broader region of highest sensitivity, possibly because it receives input from a larger number of hair cells (see also *Graphical presentation of data* in Materials and methods).

systems, only hair cells with identical, or nearly identical, polarization synapse onto a given first-order afferent neurone. In all crista segments, including segment C<sub>1</sub> from which the recordings were made, all hair cells that synapse onto a given first-order afferent neurone coincide in their direction of polarization (Budelmann, 1977; Budelmann *et al.* 1987). In the macula, which has a radial pattern of morphological polarization of its hair cells, the recordings were almost certainly made from 'peri-macular' first-order afferent neurones, which receive their input only from hair cells that coincide, or deviate by only a few degrees (average  $2.9^\circ$ ), in their direction of morphological polarization (Colmers, 1981). The recordings could also be from intra-macular first-order afferent neurones, but this would not invalidate the results. Intra-macular neurones receive their input from hair cells that deviate between  $4.4^\circ$  and  $25.3^\circ$  (average  $7.7^\circ$ ) in their direction of morphological polarization; thus, the neurones should be responsive over an angle of between  $185^\circ$  and  $205^\circ$ . Recordings from intra-macular neurones are less likely, however, because they are fewer in number than the peri-macular neurones (610 *versus* 1330; Budelmann and Wolff, 1976; Colmers, 1981) and none of the recordings showed first-order afferent units to be sensitive over more than  $180^\circ$ .

The observation that all units responded over a range of about  $180^\circ$  is of particular

interest in terms of a possible interaction between hair cells and the way in which the afferent neurones process the directional information that converges onto them from several hair cells. Lucifer Yellow staining and serial sections at the ultrastructural level show that the secondary (but not the primary) sensory hair cells of the crista and macula of octopus, cuttlefish and squid sometimes have processes at their base that intermingle with the neuronal plexus underneath the hair cells, although these processes have never been seen to make physical contact with neighbouring hair cells or even to form synapses at their ends (Budelmann and Thies, 1977; Colmers 1977; Budelmann *et al.* 1987; Williamson, 1989, 1991). Lateral inhibition has been discussed as a possible mechanism for fine tuning ('sharpening') the reception of stimulus direction in the *Octopus vulgaris* macula/statolith system (Budelmann, 1970). The results of the present experiments, however, do not give any evidence for an interaction of the hair cells in terms of a sharpening effect of their 180°-response, e.g. by lateral inhibition. In other words, in the macula, with its radial pattern of polarization of hair cells, there is a large overlap of the directional sensitivities of neighbouring hair cells. The 180° response characteristic also supports the morphological finding that only hair cells with the same direction of morphological polarization converge onto a given first-order afferent neurone. It shows, in addition, that the directional information of the hair cells is not modified by their first-order afferent neurones. Efferent input to the neurones may modify the response, but in the present experiments the brain, and thus the efferents, was removed.

The lowest thresholds (highest sensitivities) of the crista and macula hair cells/afferent units, which are displayed in Figs. 2–4 (0.12  $\mu\text{m}$  and 0.4  $\mu\text{m}$  peak-to-peak water displacement, respectively), are not the lowest thresholds obtainable for cephalopod hair cells. Williamson (1988) obtained a threshold value of 0.12  $\mu\text{m}$  for statocyst crista hair cells and Bleckmann *et al.* (1991) obtained a value of 0.06  $\mu\text{m}$  for the hair cells of the lateral line analogue of *Sepia*. In addition, the values obtained in the present experiments are unlikely to be the most sensitive values for the following reasons. First, the afferent units were selected on the basis of being easily identified in the recordings, so we preselected the largest units, not necessarily the most sensitive ones. Second, the recordings are from afferent units of crista segment C<sub>1</sub>; these are known to be approximately ten times less sensitive than afferent units of crista segment C<sub>2</sub>, because of segment C<sub>1</sub>'s much shorter cupula (Williamson and Budelmann, 1985). Third, it cannot be excluded that the attachment of the cupula, and less probably of the statolith, to the hair cells and sensory epithelia was slightly changed (damaged) during the preparation procedure.

The observation that the response characteristics of an invertebrate statocyst hair cell to stimulus direction and stimulus amplitude are identical to those of vertebrate vestibular and lateral line hair cells is of particular importance for comparative aspects of hair cell morphology and function. Cephalopod hair cells are known to differ in several structural ways that are considered to be important for the hair cell's response behaviour from the vertebrate hair cell. For example: (i) cephalopod hair cells carry up to 200 kinocilia (arranged in an elongated ciliary group) and no stereocilia, compared with the single kinocilium and several stereocilia of vertebrate hair cells; (ii) the cilia of cephalopod hair cells are all of the same length, compared with the gradation in length of the cilia of

vertebrate hair cells; and (iii) in cephalopod statocyst hair cells, tip links presumably do not occur because of the absence of a gradation in the height of the cilia (although the close membrane contacts between the tips of the cilia may function as tip links) (for references, see Introduction). Despite all these morphological differences, however, the response characteristics of cephalopod and vertebrate hair cells are identical. Thus, the cephalopod hair cell is an interesting model system, and comparative research on any of their morphological and physiological aspects can certainly contribute to a better understanding of the structure and function of the vertebrate hair cell.

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