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Published in:

Journal of Vestibular Research-Equilibrium & Orientation

Publication date:

2003

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Citation for published version (APA):

Fraser, P. J., Cruikshank, S. F., & Shelmerdine, R. L. (2003). Hydrostatic pressure effects on vestibular hair cell afferents in fish and crustacea. *Journal of Vestibular Research-Equilibrium & Orientation*, 13(4-6), 235-242.
<http://www.ncbi.nlm.nih.gov/pubmed/15096667>

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Hydrostatic pressure effects on vestibular hair cell afferents in fish and crustacea

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Revised 27 March 2003

Abstract. Following the discovery of a hydrostatic pressure sensor with no associated gas phase in the crab, and the knowledge that several systems of cells in culture show long term alterations to small changes in hydrostatic pressure, we show here that vestibular type II hair cells in a well known model system (the isolated elasmobranch labyrinth), are sensitive to hydrostatic pressure. This new finding for the vertebrate vestibular system may provide an explanation for low levels of resting activity in vertebrate hair cells and explain how fish without swim bladders sense hydrostatic cues. It could have implications for humans using their balancing systems in hypobaric or hyperbaric environments such as in aircraft or during space exploration. Although lacking the piston mechanism thought to operate in crab thread hairs which sense angular acceleration and hydrostatic pressure, the vertebrate system may use larger numbers of sensory cells with resultant improvement in signal to noise ratio. The main properties of the crab hydrostatic pressure sensing system are briefly reviewed and new experimental work on the isolated elasmobranch labyrinth is presented.

Keywords: Hydrostatic pressure, crab, elasmobranch, shark, vestibular, hair cells, sensory receptors, nerve spikes, semicircular canals, angular acceleration, balance

1. Introduction

1.1. Hydrostatic pressure sensing

Many marine invertebrates and fish show behavioral responses to hydrostatic pressure in order to regulate their depth and synchronize their behaviour to tidal cycles [1,2,16,17,22]. Hydrostatic pressure is one of the strongest factors which influence fish [21]. A possible hydrostatic pressure sensor in animals with no gas compartment remained unidentified until recently when Fraser and Macdonald discovered that thread hairs in the statocyst or balance organ of crabs are sensitive to

hydrostatic pressure, and they proposed a piston mechanism whereby differential compression of fluid inside thread hairs and the much less compressible cuticular components outside the hairs could lead to nanometer level displacements sensed by mechanoreceptors [14]. In their model, assuming cuticle was incompressible and that the fluid contents had a compressibility comparable to seawater ($44 \times 10^{-6} \text{ Bar}^{-1}$), a 1 Bar (100 kPa) pressure caused a volume change inside each $2 \mu\text{m}$ diameter, $400 \mu\text{m}$ long thread hair which led to a 17.6 nm displacement transmitted to the dendrites of two bipolar neurones via the chorda (cuticular linking rod). An above threshold 1° angular displacement of the thread hair would cause a 17.5 nm linear displacement of the chorda. Since known thresholds for mechanoreceptors are less than 1 nm, the mechanism can account for the 5–10 mbar (500–1000 Pa) thresholds to hydrostatic pressure step changes known for crustacea.

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In the crab, spontaneous and angular acceleration derived activity in thread hair receptors shows a clear excitatory relationship, or an inhibitory relationship with slow (10–750 minute period, 1–50 kPa peak to peak amplitude) cycles of hydrostatic pressure. Positive and negative relationships of thread hair spike rates with hydrostatic pressure in the crab, *Carcinus* are thought to be derived from the two directional classes of thread hair units [10,12], supporting the piston mechanism, and precluding metabolic linked effects.

The sensitivity of adult crabs to hydrostatic pressure, and the low thresholds found, are hence adequately explained. This also offers an alternative to Digby's hypothesis which proposes that transcuticular potentials are affected by pressure acting on a thin layer of nascent hydrogen, and somehow transduced by unknown sensory nerves [6,7]. Statocyst interneurons in a crab under water do not code hydrostatic pressure directly, but spectral analyses of their spike activity following pressure cycles shows that the cells may carry hydrostatic pressure information via central pathways [10]. Recordings from statocyst interneurons taken from crabs out of water show a more direct influence of hydrostatic pressure [11].

2. Vertebrate hair cells and hydrostatic pressure

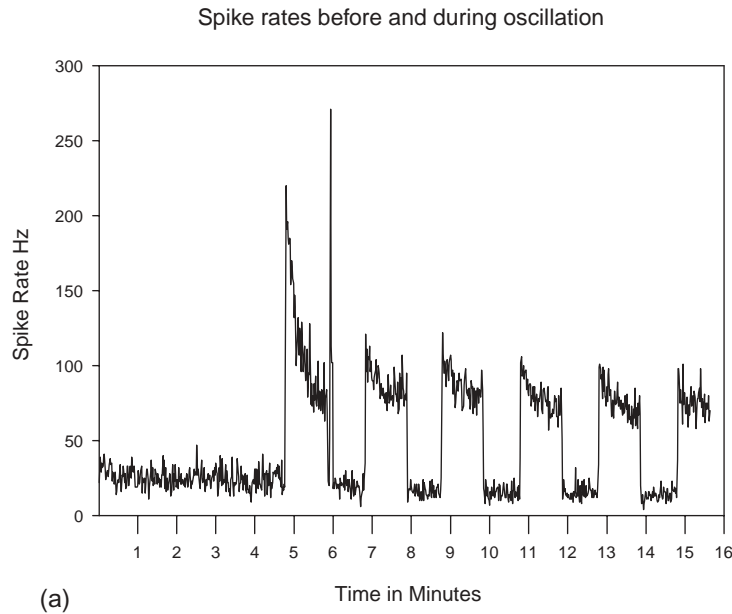
Several systems of cells in culture have been shown to respond by altered cell proliferation rates or in other ways, to extremely small hydrostatic pressure changes, in the order of a few centimeters of water pressure (see review [20]). A recent attempt to find length changes or trans-membrane voltage changes in isolated cochlear hair cells following small elevations in hydrostatic pressure failed to find significant changes, but small effects may have been masked by continuous length changes following isolation of the cells [4]. During a long term study from a variety of semicircular canal units and tonic and phasic statoreceptors in the bullfrog, the influence of a "sudden increase in pressure", (which could refer to barometric pressure) caused a modification of the firing rate [3].

We report here that a well known model hair cell system, the isolated elasmobranch labyrinth [18], shows altered resting and angular acceleration derived activity following steps (14 kPa) and sinusoidal pressure cycles (30 kPa amplitude, 15 minute period). The result carries implications both in terms of the ability of animals to sense hydrostatic pressures, but also may point to the importance of hydrostatic pressure as an adequate

stimulus in auditory systems. This could act as a basis for depth sensing mechanisms in fish and explain the signalling value of low level resting activity in hair cells. A preliminary account of this work on the dogfish system has been published [9]. Instead of cuticular thread hairs, vertebrates have hair cells in their balancing and auditory systems, so the piston mechanism could not apply directly to explain how fish without swimbladders could sense hydrostatic pressure.

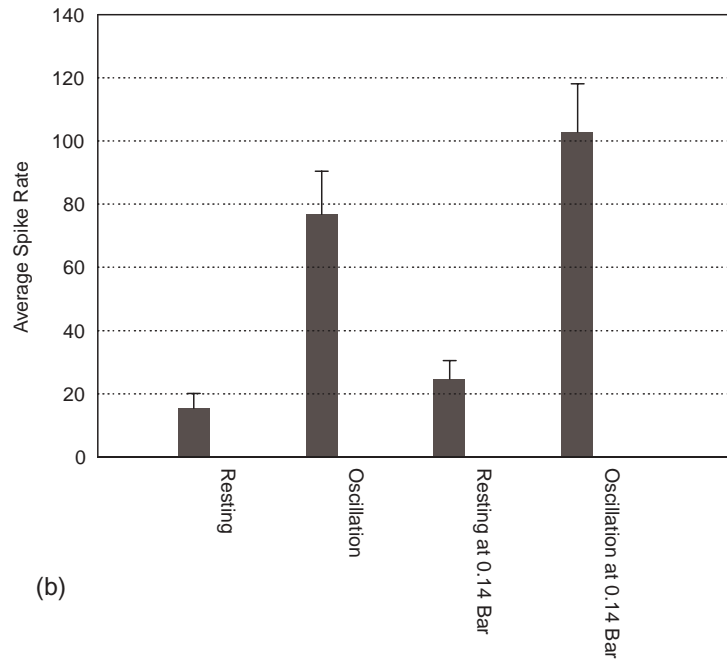
3. Methods

7 dogfish, *Scyliorhinus canicula* (weighing around 1kg) were killed by decapitation, and prepared as in [19] but with the part of the cranium held in a small perspex dish using tacky wax. The branch of the eighth nerve associated with the horizontal canal was exposed and extracellular recordings made using teflon coated silver wire electrodes. Nerve spikes were amplified using a WPI Dam 50 amplifier and passed through filters (Digitimer, Neurolog NL125; Bandpass 800 Hz to 2 kHz), to a spike trigger (Neurolog, NL200) or digitized and recorded using an Instrutech VR100 interface and video recorder. The preparation was covered with a lid of damp tissue paper to prevent desiccation and to minimize effects of air movement. It was further mounted on an Inland DC Servomotor system inside a pressure chamber. This chamber consists of top and bottom base plates made out of half inch steel machined to fit into the ends (with an O-ring seal) of a 30 cm diameter, 30 cm long, 1 cm wall thickness plastic gas pipe. Top and bottom plates are clamped on either end of the cylindrical pipe by 6 screwed and bolted rods. The top plate has an air input, a safety valve and a pressure gauge. The bottom plate has seals for the electrical connections. This system was controlled using custom Z80 machine code software running on a Nascom 3 microcomputer as described in [15]. This allowed series of 64 oscillations of the semicircular canals in the horizontal plane at a defined amplitude and frequency. Hydrostatic pressure was controlled using an E/P converter (RS729-486) under computer control. A set sequence was used consisting of up to 1 hour at atmospheric pressure (taken as 0 Bar) followed by a step to 14 kPa (0.14 Bar), and a further 0.5 hour before giving 4 sinusoidal pressure cycles of a further 30 kPa amplitude and 15 minute period (see Figs 2a and b). Starting about 15 minutes before the initial step of pressure, at 2 minute intervals, bouts of oscillation around the vertical axis, each consisting of 64 cycles at 1 Hz and 70



(a)

Average Spike Rates and Standard Deviations Before and After Pressure



(b)

Fig. 1. Typical extracellular spike rates (counts per second) from a right vestibular system of dogfish. a. before and during a series of 64 oscillation cycles every two minutes. Note transients. b. Average spike rates and standard deviations during resting periods and during the bouts of oscillation before application of hydrostatic pressure and during application of hydrostatic pressure.

degrees amplitude were given. TTL pulses from the spike trigger were averaged on-line using 10msec bins, and average histograms stored and used to calculate

spike counts and the phase of the histogram peak. Alternatively recorded sequences were played back utilizing four spike triggers set to select different units

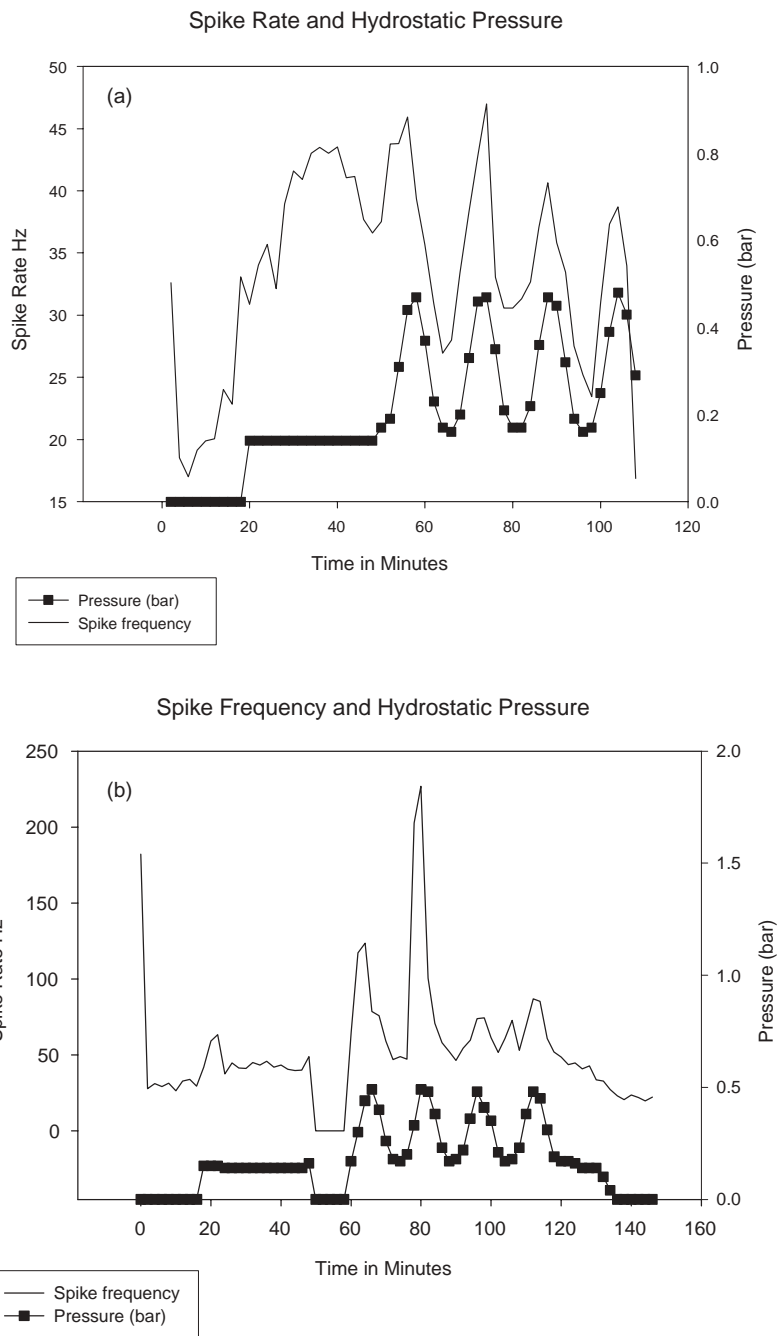


Fig. 2. a. Spike Rate from horizontal canal units from the right vestibular system of dogfish during bouts of 64 cycles of oscillation (1 Hz, 70°) every 2 minutes before a 0.14 bar step of pressure followed 30 minutes later by four 15 minute sinusoidal pressure cycles. b. A similar sequence from the left vestibular system of the same fish. A tube blew off causing an unplanned pulse of reduced pressure which clearly enhanced the response to the pressure cycles.

or small groups of units and spikes were counted in 1 second or 12 second bins using a National Instruments TIO20 interface and custom made software. Temperature changes were restricted to less than 0.5°C per

hour.

For the recordings from crab thread hairs, isolated crab statocysts were set up and tested as described by [13] on the apparatus described above or using pres-

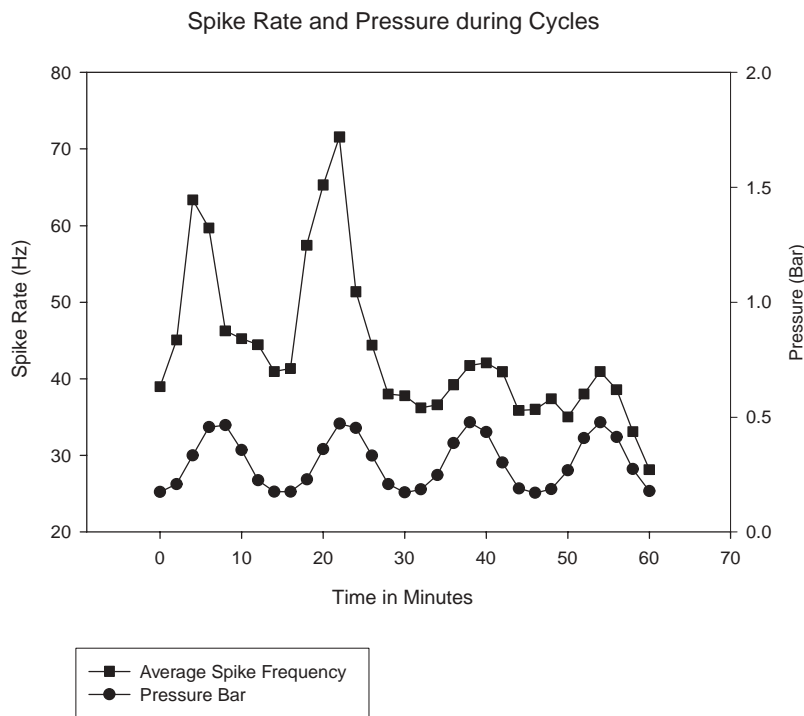


Fig. 3. Average spike rate from 6 preparations during the imposed hydrostatic pressure cycles. The first two cycles were greater than the last two, showing a partial response to the step up to the elevated average value during the cycles.

sure chambers connected via a buffer air reservoir to a tide machine which provided a flow of seawater at different pressures according to the vertical height of a water reservoir. The height of this was controlled with a computer controlled stepper motor, to generate steps or sinusoidal hydrostatic pressure changes [5].

4. Results

4.1. Dogfish

In the resting state, low levels of spontaneous activity were observed in individual units. Usually spike discriminators were set to record from small sets of units. At the start of bouts of sinusoidal oscillation (64 cycles, 1 Hz), spike counts per second increased markedly. They then rapidly declined after the initial cycles. Although adaptation was observed in the subsequent bouts of oscillation (repeated every two minutes), it was always much less than when left for a long time. In these subsequent bouts, average spike counts per cycle of oscillation were less. Spontaneous activity in small sets of dogfish hair cell units between bouts of sinusoidal oscillation was lower than the resting level before application of any bouts (Fig. 1a).

Following application of pressure there was a significant increase in resting spike rates (Mann-Whitney U-test). Figure 2a shows a typical recording during a step of pressure and during a series of four cycles of pressure varying sinusoidally. Units responded unidirectionally to oscillation at 1 Hz, and typically the number of spikes per cycle of oscillation showed initial rapid adaptation and then a slow decline over a period of several hours. In one case, during application of hydrostatic pressure, a tube came off, causing a step decrease in hydrostatic pressure to atmospheric inside the chamber for almost 10 minutes. On reapplication of the pressure, a much larger peak in thread hair activity than usual was noted, and the peak to the second cycle of hydrostatic pressure was greater again, implying a response to the greater step from 0 bar instead of from 0.14 bar at the start of the cycles (Fig. 2b).

Spike rates either increased significantly (Mann-Whitney U-test) following the initial step change in pressure, when stable levels had been reached, or if the step was given when levels were still adapting following setup, the rate of fall of spike rate was noticeably less or turned into a net rise. Cyclical changes matching the imposed 15 minute period hydrostatic pressure cycle were apparent during the pressure cycles, but

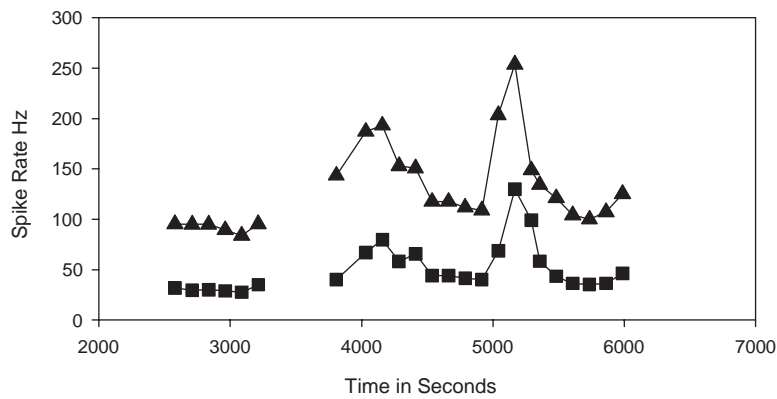


Fig. 4. Both oscillation derived (■) and resting activity (◆) co-varied with hydrostatic pressure, showing data during 0.14 bar and during the first two cycles of pressure.

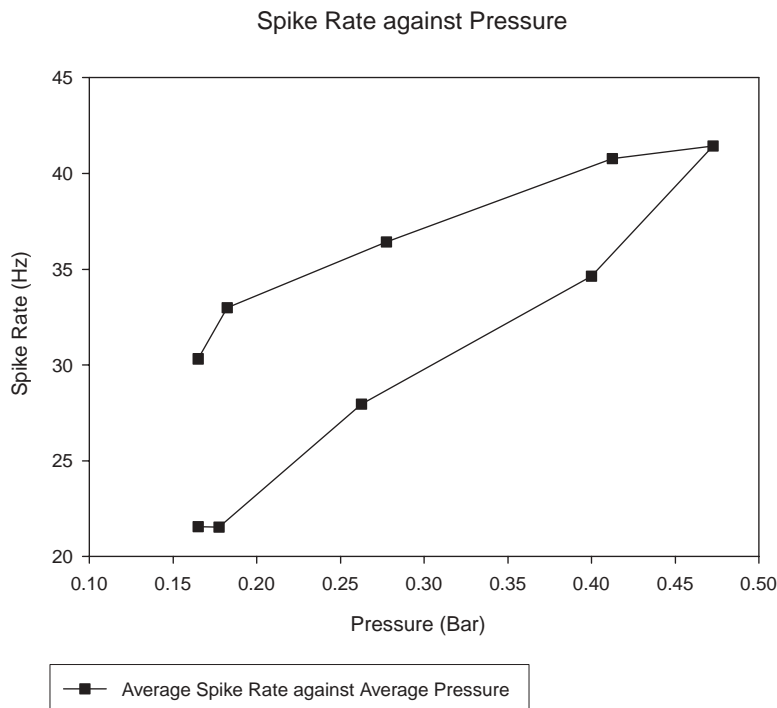


Fig. 5. Spike rate against hydrostatic pressure for a series of cycles of pressure. Significant positive going relationships were found. Negative relationships as found for the crab were never found for dogfish.

not during the preceding periods. This was confirmed using autocorrelation and maximum entropy spectral analysis [8]. Figure 4 shows the average response from units from 6 dogfish during the cycles. Both resting and oscillation derived activity varied with hydrostatic pressure.

When spike rate was plotted against hydrostatic pressure either no relationship or a positive relationship was observed (Fig. 5). Note the hysteresis in the response.

A negative relationship which was shown in many crab statocyst neurones given the same hydrostatic pressure regime was never observed. There was no significant relationship between spike rate and rate of change of pressure. Spontaneous activity and oscillation driven unit activity both showed significant increase during pressure steps or cycles (Fig. 5). Ascending and descending changes in spike rate during imposed pressure changes were gradual as shown in Fig. 6.

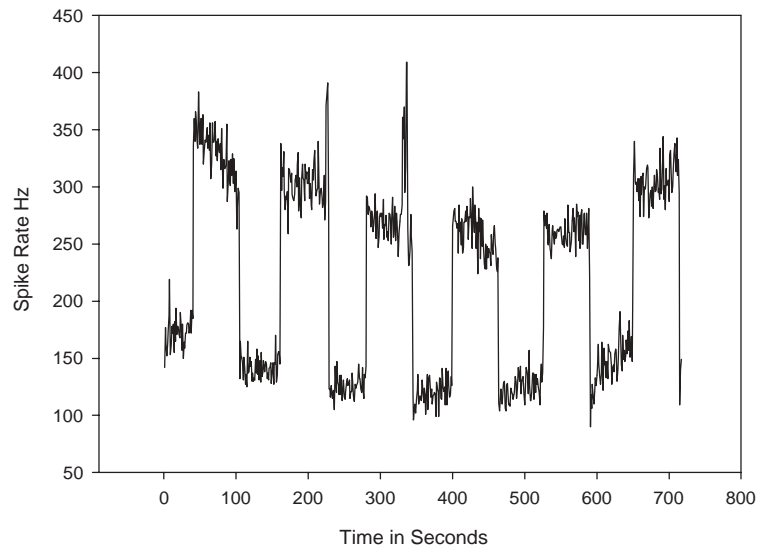


Fig. 6. Spike rate at the descending phase, turning point and ascending phase of the pressure cycle. The profile of spike rate during the bouts of oscillation (elevated rates) is altered from the normal which shows rapid adaptation during the first few seconds (see Fig. 1a). The pressure cycle can be seen to be altering the response extremely gradually.

5. Conclusion

This study is the first time that hydrostatic pressure sensitivity has been demonstrated in a vertebrate hair cell system. It is likely that differential compression of cartilaginous components, cupula and hair cells leads to nanometer scale displacements activating the hair cells directly. This finding has implications for transduction mechanisms in a variety of hair cell types including cochlear hair cells where complex electromechanical mechanisms are known. Although no analogous structure to the long, narrow thread hair in the crab is known in the vertebrate system, precluding a piston mechanism, greater numbers of cells would allow averaging to increase signal to noise ratios. Unlike units with high levels of resting activity, irregular units with very low rates of resting activity clearly could not code much information during angular displacements in a direction which would inhibit their activity. An alternative function is suggested here whereby they could code hydrostatic pressure, by integrating information over long time scales.

Acknowledgements

We would like to thank NERC and The Royal Society of London for providing equipment used in this study, and NERC for providing a studentship for SFC.

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