An angular acceleration receptor system of dual sensitivity in the statocyst of *Octopus vulgaris*[1]

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Summary. The angular acceleration receptor system of the statocyst of *Octopus vulgaris* can be classified morphologically into two sub-systems on the basis of cupula form and size[8]. Electrophysiological experiments demonstrate that these two subsystems differ in their sensitivity ranges; one is appropriate for the detection of low and the other for the detection of higher angular accelerations.
Key words. Octopus vulgaris; statocyst; angular acceleration receptor system.

Cephalopods have the most sophisticated equilibrium receptor system of all the invertebrates. This statocyst system is of particular interest because it shows a high degree of convergence with the vertebrate vestibular apparatus and similarly has receptor organs for the detection of linear as well as angular accelerations[2–8]. The statocysts of the different cephalopod genera show a wide range of complexity and levels of differentiation and it has been suggested that such differences can be correlated with the different forms of locomotion employed by the animals, e.g. *Octopus* usually crawls slowly along the bottom of its rocky habitat, but it is also capable of swimming by jet propulsion[9–11]. In the statocyst of *Octopus vulgaris*, the receptor epithelium (crista) of the angular acceleration receptor system runs like a ridge over three planes almost perpendicular to one another. This crista is subdivided into nine sections (C1–C9), to each of which a sail-like cupula is attached[3]. The odd-numbered crista sections (C1, C3, C5, C7, C9) differ from the even-numbered ones (C2, C4, C6, C8) in their morphology and neuronal organization[8]. The most obvious differences occur in the form and size of the cupulae. In the odd crista sections, the cupula (type I) is small (maximum height: ~ 165 µm; maximum width, ~ 275 µm), and it has a wide area of attachment to the underlying receptor hair cells and the adjacent tissue (fig. 1)[8]. In contrast, in the even crista sections, the cupula (type II) is large (maximum height: ~ 480 µm; maximum width: ~ 230 µm), but its area of attachment to the receptor cells is only small (approximately ¼ of that of type I; fig. 1)[8]. These differences in cupula morphology do not arise from fixation artefacts[8]. Any angular acceleration or deceleration stimuli applied to these crista/cupula systems result – because of inertia of the surrounding endolymph fluid – in a deflection of the cupula relative to the crista ridge and thus in an excitation or inhibition of the receptor hair cells, depending on their polarization[3]. The differences in cupula size and cupula attachment to the crista ridge consequently suggest different functional properties of the two crista/cupula systems.

Material and methods. The activity of afferent units from the crista sections C1 and C2 was recorded extracellularly with a suction electrode in isolated head preparations of 31 *Octopus vulgaris*. The electrode tip was introduced through a small hole cut in the cranial cartilage just dorsal to either crista section C1 or C2, and placed lightly on the axons of C1 or C2 afferent units. The course of these axons is known from neuroanatomical studies[8]. The preparation was fixed in its normal upright position on an earth-horizontal rotating arm. Forward (head-down) and backward (head-up) sinusoidal oscillations around the animal's transverse axis (i.e. the y-axis, which is appropriate for stimulation of C1 and C2 receptor cells[3]) could then be applied. The rotation device was controlled by a servo-motor system driven by a function generator. The procedure consisted of locating a C1 or C2 single unit and recording the response to sinusoidal oscillations around the normal position at up to 10 selected frequencies within the range of 0.01–1.6 Hz and with a constant amplitude of 12°. Although in the preparation the blood supply was destroyed and not replaced by perfusion, the responses remained constant from 10 min (earliest recording) up to 6 h after decapitation.

Results. In crista section C2 (with large cupula type II) it was found that, with the preparation stationary in the normal upright position, 62% of the C2 units displayed resting activity. In contrast, in crista section C1 (with small cupula type I) none of the units was found to show resting activity. The response of an afferent unit, from either crista section C1 or C2, to sinusoidal oscillation consisted of an increase in activity with movements in the one direction (head-up) and a decrease in

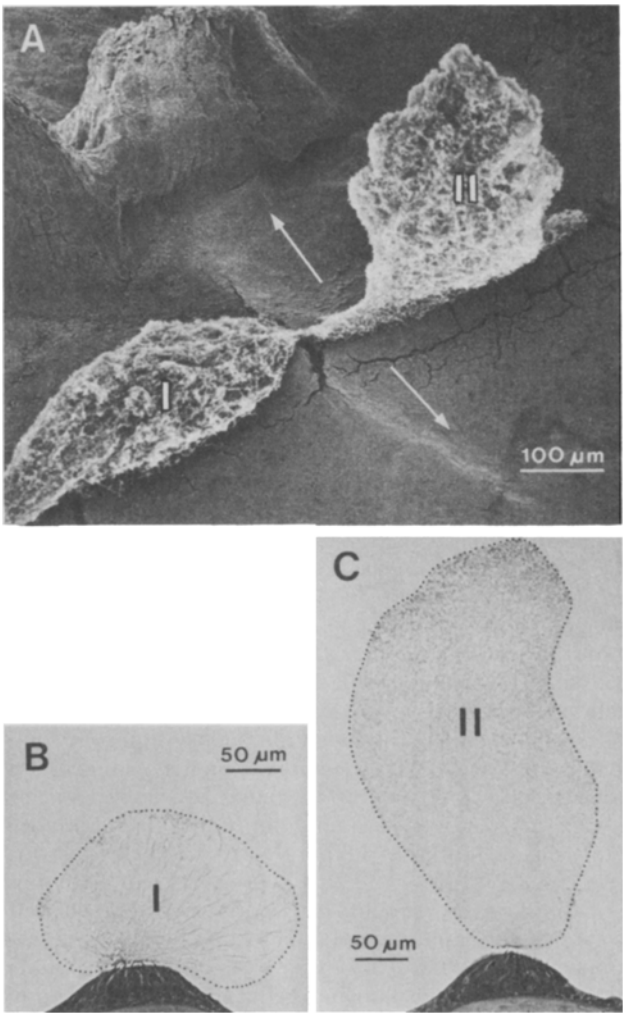

Figure 1. *A–C* Scanning electron micrograph (*A*) and light microscopical cross-sections of cupulae (*B, C;* outline highlighted by dots) of the angular acceleration receptor system of the statocyst of *Octopus vulgaris*. The cupulae are sail-like structures that move like swinging doors. Cupula type I (to the left) is attached to the odd crista sections (C1, C3, C5, C7, C9); it is small and has a wide base in contact with the kinocilia of the receptor hair cells and with the adjacent tissue. Cupula type II (to the right) is attached to the even crista sections (C2, C4, C6, C8); it is large and has a narrow base. Angular acceleration stimuli will result in a movement of the surrounding endolymph fluid relative to the crista (arrows) and thus cause the cupulae to be deflected in a direction opposite to that of the acceleration. This deflection, in turn, excites or inhibits the receptor hair cells, depending on their polarization.

activity (or no activity) with movements in the opposite direction (head-down) (e.g. fig. 3, inset). Thus all units increased their activity in response to a ventral cupula deflection and decreased their activity in response to a dorsal cupula deflection. This pattern of discharge is to be expected from the ventrally polarized receptor units in the crista[3,12] (i.e. the secondary sensory hair cells and their first-order afferent neurones[8]).

However, not all units responded to the entire range of stimulus frequencies employed. For most units there existed a low frequency threshold below which no evoked response could be observed. These individual response thresholds of C1 and C2 units are plotted in figure 2. It is apparent that, although there is some overlap, the C2 thresholds are about 10 times lower than the C1 thresholds. Most of the C2 units therefore are more sensitive, and thus appropriate for the detection of lower angular accelerations, than the C1 units.

The peak firing rate for each C1 and C2 unit was determined at the different stimulus frequencies (fig. 3). Both types of units increased their activity with increasing stimulus frequency, with the C1 units showing lower levels of peak activity than the C2 units for the same stimulus. In addition, 40% of the C2 units were found to approach saturation of peak firing at the upper stimulus frequencies (> 1.0 Hz). None of the C1 units were found to show a saturation of their response when tested at frequencies of up to 1 Hz and it is therefore probable that the C1 units are able to operate at higher stimulus frequencies than the C2 units.

Discussion. The experiments demonstrate differences in response thresholds between afferent units of the two crista sections C1 and C2. Separate recordings from the other crista sections C3–C9 are difficult to obtain because the afferent fibers from several crista sections join and then run together. However, there is no reason to believe that the functional properties of the other odd and even crista sections are different from those described for C1 and C2.

The difference in response thresholds between the units of the two crista sections C1 and C2 could result from the difference in cupula size (type I and type II) as well as from the difference in cupula attachment to the crista ridge. Any given angular acceleration will cause a larger cupula deflection, and thus a larger stimulation of the receptor cells, in the system with the large cupula type II (i.e. C2, with the small area of cupula attachment to the crista ridge) than in the system with the small cupula type I (i.e. C1, with the wide area of cupula attachment).

A separation of an angular acceleration receptor system into two subsystems of different sensitivity has never been described so far in the animal kingdom; in the *Octopus* it can be understood as an adaptation to the animal's two forms of locomotion. Slow crawling along a surface requires a receptor system of high sensitivity at low frequencies (i.e. crista/cupula type II), whereas fast swimming by jet propulsion, which in squids of comparable size can produce a maximum linear acceleration of about 32 m s^{-2} (3.3 g)[13,14], requires a system of lower sensitivity appropriate to higher frequencies for rapid turns (i.e. crista/cupula type I). Furthermore, the results support the observation[11] that slowly moving cephalopods have statocysts with angular acceleration receptor systems of large dimensions, whereas fast moving cephalopods have systems of small dimensions.

Although in the vertebrate vestibular apparatus there is no comparable morphological division of the angular acceleration receptor system into two separate subsystems, there are obvious differences in the sensitivities of the vertebrate semicircular canal afferents in that the receptor hair cells in the center of the crista are more sensitive to a given angular acceleration than those in the crista periphery[15,16]. This differential sensitivity in a single crista/cupula system has been explained by the shape and movement of the cupula. The cupula increases in thickness from its center to its periphery and because it is fixed to the ampulla wall and thus forms a tight diaphragm, any endolymph movement will result in a cupula movement which has its maximum over the center of the crista ridge and decreases towards the periphery[17–19].

Furthermore, in vertebrates, where there are two types of receptor cells, the type I hair cells have a higher sensitivity than the type II hair cells, independent of their position in the crista[15,20,21]. Experiments in progress will show whether such differences at the level of the physiological properties of the receptor cells also exist in the *Octopus* system.

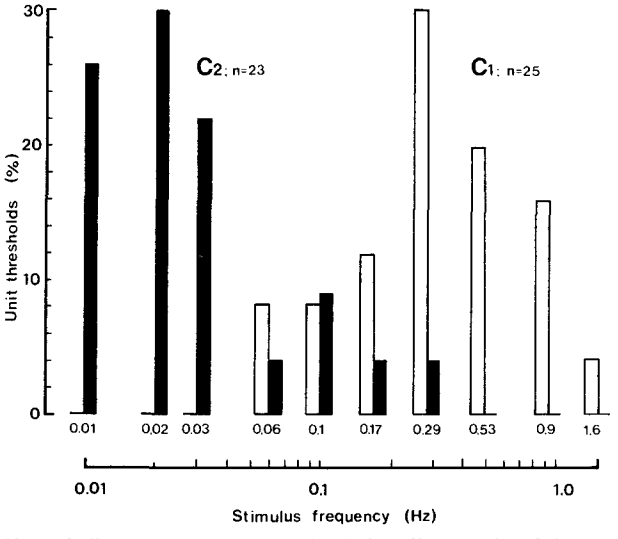

Figure 2. Frequency response thresholds for afferent units of the two different crista/cupula systems of *Octopus*. The responses of the afferent units in crista section C1 (with the small cupula type I) and C2 (with the large cupula type II) were tested during sinusoidal oscillations (amplitude 12°) at 10 different stimulus frequencies. The percentage of C1 and C2 units having their threshold at each of the test frequencies is shown, with C1 values given as open columns and C2 values as filled columns.

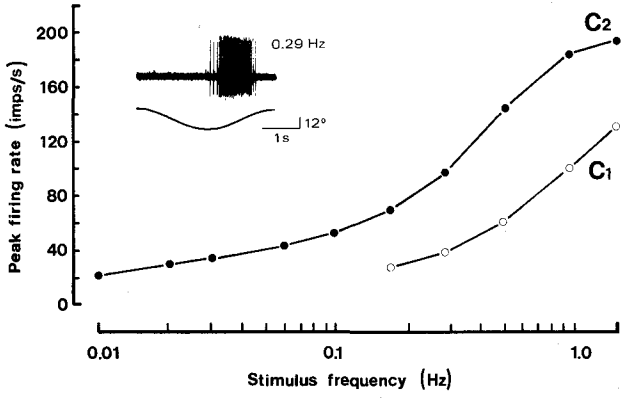

Figure 3. Peak firing rate (impulses per s) of a C1 and a C2 afferent unit during sinusoidal oscillations (amplitude 12°) plotted against the stimulus frequency (logarithmic scale). Note that the C1 unit shows no response below 0.1 Hz and that the C2 unit begins to saturate at the higher stimulus frequencies. The inset shows a C2 afferent unit responses at 0.29 Hz. The upper trace shows the activity of the unit and the lower trace the applied sinusoidal oscillation.

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Hemodynamic studies in a parabiotic model of portal hypertension

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Summary. Splanchnic and systemic hemodynamic studies were performed in a rat model of parabiosis and portal hypertension. A portal hypertensive and a normal rat were surgically united side to side. A hyperdynamic circulation, characterized by increased cardiac index (413 ± 26 vs 318 ± 23 ml·min^{-1}·kg^{-1}; $p < 0.05$) and portal venous inflow (9.61 ± 1.29 vs 6.33 ± 0.36 ml·min^{-1}·100 g b.wt^{-1}; $p < 0.05$), was found in all the portal hypertensive rats but not in the normal parabiotic partners. These results do not support the existence of a transferable humoral factor mediating the hyperdynamic circulatory state of chronic portal hypertension.
Key words. Parabiosis; portal hypertension; hyperdynamic circulation.

Vasoactive humoral factors have recently been suggested as possible mediators of the hyperdynamic circulatory state[2,3] observed in portal hypertensive patients[4,5] and experimental models of portal hypertension[6,7]. Chronic plasma exchange between portal hypertensive and normal animals may induce this syndrome in the normal animal and will therefore confirm the existence of a humoral factor. To explore this possibility we performed splanchnic and systemic hemodynamic studies in a model of parabiosis in which a portal hypertensive and a normal rat were united. Parabiosis, the union of two living individuals through a capillary bed, has been a profitable tool in evaluating humoral transferable effects especially in endocrine studies[8]. Previous studies in this model have shown that plasma exchange between two united rats ranged from 0.47 to 1.04% of one animal's plasma volume per min[9].

Material and methods. Male, Sprague-Dawley litter mate rats were used. Portal hypertension was induced by partial ligation of the portal vein (PVL). The operative procedure for portal vein constriction has been described in detail[10]. In brief, the portal vein was isolated and the stenosis was created by a single ligature of 3-0 silk around the portal vein and a 20 gauge blunt-tipped needle. The needle was then removed and the portal vein was allowed to reexpand distal to the stenosis. 1 week later parabiosis was performed by uniting a portal hypertensive and a normal rat according to the surgical technique described by Bunster and Meyer[11]. The rats were anesthetized by ketamine 100 ml·kg^{-1}. After appropriate shaving, an incision was made from the base of the ear to the tail. Then each pair of rats was joined by sutures of surgical silk (No. 3-0) through scapula and musculature of abdomen and thigh. Matching skin edges were joined with metal autoclips. Each pair was housed in an individual plastic cage and allowed free access to rat chow and water. Weight gain was an indicator of healthy animals in a successful parabiotic union. 10 parabiotic pairs were prepared. 4 weeks after the parabiosis, hemodynamic studies were performed under ketamine anesthesia (100 mg·kg^{-1}). Four pairs were excluded earlier because of failure to thrive. One pair was excluded because of severe bleeding during the hemodynamic study. Five pairs were successfully studied. Cardiac output, organ blood flow and portal systemic shunting (PSS) were determined by a radioactive microsphere technique which was described in detail in previous communications from our laboratory[12]. Arterial pressure and portal pressure were also measured. Portal venous inflow (PVI) was the sum of arterial blood flow of the stomach, intestine, spleen, pancreas and mesentery. The venous outflow of each of these organs is into the portal venous system, PVI therefore, represents the total splanchnic arterial inflow entering into the portal system. In order to confirm the existence and the amount of the exchange of plasma, 0.5 ml of a 20% Evans blue solution was injected into the penile vein of the portal hypertensive rat. 15 min later arterial blood samples were taken from the two rats. The serum was separated and stored at −70 °C until dye concentration was measured. In each pair, plasma exchange was calculated from the formula:

$$r = \frac{Cn}{Cp} \times \frac{100}{15}$$

where r = rate of exchange in 1 min expressed as a percentage of one animal's plasma volume, Cn and Cp = Evans blue concentration in normal and portal hypertensive rats respectively. At the end of the experiment the rates were sacrificed by a bolus injection of KCl, separated and weighed individually.

Data is expressed as mean ± SEM. Statistical analysis was performed by the unpaired t-test.

Results and discussion. At the time of the study the weight of the PVL and the normal rats was similar 326 ± 31 vs 344 ± 16 g. The rate of plasma exchange in 1 min was 1.4 ± 0.3% with a range of 0.5 to 2.3%. The hemodynamic data are depicted in the table. Previous studies from our laboratory have shown that portal vein constriction in the rat provides a reproducible model of portal hypertension in which portal systemic shunting and a hyperdynamic systemic and splanchnic circulation develop in a