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TOUCH-PLATE AND STATOLITH FORMATION IN GRAVICEPTORS OF EPHYRAE WHICH DEVELOPED WHILE WEIGHTLESS IN SPACE

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Abstract

Ultrastructural studies of the statocysts and touch-plates of graviceptors (rhopalia) of *Aurelia* ephyrae revealed that (1) touch-plate hair cells are present; and (2) cytoplasmic strands from the hair cell bases extend from the neurite plexus to touch similar strands from the lithocytes. This close association of hair cell neurites and statocysts may have important implications regarding the transmitting and processing of positional information with respect to the gravity vector.

Graviceptors of ephyrae which developed while weightless in microgravity were compared with controls at the ultrastructural level. We found that hair cells of ephyrae which developed in microgravity had fewer lipid droplets in the large spaces near their bases as compared with 1 *g* controls. In the ephyrae from the first microgravity experiment, hair cells had more large apical vacuoles with filamentous content than were found in hair cells of ephyrae from the second experiment and controls. The neurite plexus and the network of cytoplasmic strands extending to the statocysts were not different in microgravity-developed ephyrae from controls. Behavioral differences in swimming and orienting in ephyrae in microgravity and controls (reported earlier) were not explained by morphological differences in the hair cells of the touch-plates or the statocysts, although functional differences apparently occurred.

Key Words: Graviceptors, touch-plates, hair cells, statocysts, statoliths, ephyrae, development, neurites, weightlessness, microgravity.

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Introduction

Little research has been done previously on the cellular organization of *Aurelia* ephyra graviceptors. In these animals, eight graviceptors (rhopalia) are located at the tips of their arms between their lappets. The graviceptors have statocysts at their tips and a touch-plate adjacent to the statocyst. In ephyrae newly-released from their strobilae, statolith formation in the lithocytes of statocysts under normal and experimental conditions was described by Spangenberg (1976, 1986) as was the ultrastructural morphology of the mechanoreceptor (MR) cells (Spangenberg, 1991). These newly-released ephyrae did not have an organized touch-plate. The touch-plate is found in medusae (and older ephyrae) adjacent to the statocyst on the underside of the graviceptor (when the oral side of the organism is facing upward). The tiny ephyrae lack a hood, which is found in medusae (Hyman, 1940), over the touch-plate area of the rhopalium.

Hyman (1940) described the importance of the statocyst for equilibrium of medusae and more recently, Hundgen and Biela (1982) stated that the statolith together with the touch-plate constitutes the static sense organ. Chapman and James (1973) reported that the touch-plate has static flagella and, no doubt, is involved in the appreciation of the tilt of the medusa. In *Aglantha*, Mackie (1980) concluded that the statocysts (a term, which in this organism, includes statoliths and hair cells) are graviceptors and that input from them is necessary for producing the motor responses responsible for righting during each swimming cycle. He states that sensory input from the statocysts affects the direction of locomotion but the mechanism is unclear.

For the first time, *Aurelia* polyps were induced to metamorphose (through strobilation) and to form ephyrae in a microgravity environment. The purpose of the jellyfish experiment which flew on NASA's (National Aeronautics and Space Administration) Spacelab Life Sciences (SLS-1) shuttle flight was to determine whether ephyrae, and especially their graviceptors, could develop during a nine day mission in microgravity. Ephyrae

with graviceptors developed both during this mission and during a second flight experiment on shuttle mission IML-2 which provided fourteen days of microgravity to the metamorphosing jellyfish. In the presence of 1 *g*, ephyrae tend to swim upward and, when not pulsing, to drift downward with their mouths oriented downward. During both flight experiments, ephyrae that developed in microgravity tended to swim in loops and circles and were stationary when not pulsing (Spangenberg, 1995). In addition, some ephyrae did not pulse and swim normally upon return to Earth (Spangenberg *et al.*, 1994a). In an effort to understand whether abnormal behavior was caused by abnormal ephyra graviceptor development in microgravity, we have investigated the regions of the rhopalia most directly related to graviception, the touch-plate and statocyst areas.

Ephyrae from the shuttle flight experiments were recovered after being free from their strobila a longer time (48-120 hours) than the newly-released ephyrae studied previously (Spangenberg, 1991). These ephyrae have rhopalia with a touch-plate whose sensory cells are described in this paper. We use the term "hair cells" to define only those touch-plate cells which have a hair bundle of microvillae and stereocilia (mv/st) of stairstep lengths associated with the kinocilium. The stereocilia are distinguished from microvillae by the presence of a core of fine filaments which extend into the cytoplasm as rootlets. The term "hair cell" was previously applied to jellyfish by Budelmann (1988) who stated that, in invertebrates, gravity receptor systems having hair cells with a single kinocilium are known only in the free-swimming cnidarian medusae. Arkett and Mackie (1988) also applied the term "hair cell" to cells in *Aglantha digitale* which have a single kinocilium surrounded by microvillae of stair-step lengths. Sensory cells in the rhopalium of ephyrae which have a single motile kinocilium which is surrounded by microvillae of nearly equal length are called mechanoreceptor (MR) cells in this paper. This term was used previously by Hundgen and Biela (1982) to describe medusa touch-plate sensory cells and by Spangenberg (1991) to describe mechanoreceptor cells on the rhopalia which are not hair cells.

Methods

Aurelia polyps used for this study were collected in Norfolk, VA and cultured in artificial sea water (ASW) (Spangenberg, 1965) for at least six years. The organisms were fed newly-hatched *Artemia* once weekly and transferred into clean ASW within 24 hours after feeding.

The SLS-1 experiment

In preparation for the SLS-1 flight experiment, twenty-five thousand polyps were mixed together and subsequently divided into flight and ground control groups. These polyps were further subdivided into groups which were induced to metamorphose with 10^{-5} M iodine (Spangenberg, 1967) 24 or 48 hours pre-flight and groups which were induced to metamorphose 8 hours after launch in-flight (the ultrastructure studies were done on the in-flight induced and in-flight fixed groups, with the one exception of a 24 hours pre-flight induced and immediately post-flight fixed group). All of the animals were rinsed 12 times in ASW prior to dividing them into groups of 100 in 150 ml of ASW for the pre-flight induced groups and groups of 66 in 100 ml for the in-flight induced groups. A 1.5 ml of solution per animal and a 1:3 ratio of air to solution were available for both the flight animals and their controls during the experiment. Ground control organisms were treated in a comparable manner as the in-flight organisms throughout the experiment.

Chemical delivery systems (CDSs) developed for the SLS-1 experiment by NASA were used for the introduction of iodine and fixative to the jellyfish. Kapak bags (Kapak Corp., Minneapolis, MN) of polyester with polyethylene lining were modified for attachment to syringes via plastic housings (Rossberg and Spangenberg, 1987). These CDSs were carefully cleaned and tested for biocompatibility prior to use. Three days pre-flight, the inducement and fixative chemicals were placed in small plastic baggies inside the syringe barrels of the CDSs. In some groups, iodine was injected into the bags containing polyps 8 hours after launch to achieve a final concentration of 10^{-5} M. Glutaraldehyde (3% final concentration) and cacodylate buffer (0.1 M final concentration) were injected simultaneously into the bags containing ephyrae and end pieces of strobilae on the eighth day of the mission while in-flight. Throughout the flight, the CDSs were contained within inner and outer plastic bags to achieve triple containment (for in-flight safety) and packed in an aluminum box at 28°C (after being at 22°C for eight hours).

The jellyfish were retrieved within 3 hours post-flight and those organisms which had been fixed in space were subjected to an additional 2 hours in fresh glutaraldehyde in buffer, rinsed, and transferred to cacodylate buffer for storage. The organisms which had been induced 24 hours pre-flight were fixed as soon as possible post-flight in 3% glutaraldehyde, rinsed four times and stored in buffer. All of the ephyrae were later post-fixed in 1% osmium prior to preparation for viewing with the transmission (TEM) and scanning electron microscopes (SEM). Those samples which were prepared for the TEM were in osmium for 1 hour at 4°C whereas those prepared for the SEM were exposed to osmium for only five minutes. The ephyrae were

further prepared for SEM examination by dehydration in ethyl alcohol, critical point drying, and coating with gold-palladium. They were viewed with a Philips 515 SEM (Philips Electronic Instruments, Mahwah, NJ). Ephyrae selected for TEM studies were removed from buffer, dehydrated with ethanol, embedded in Polybed 812 (Polysciences Inc., Warringon, PA), sectioned with a Porter-Blum microtome (Ivan Sorvall Inc., Norwalk, CT) and viewed with a Philips 301 TEM and a JEOL JEM-1200EX II TEM (JEOL USA, Peabody, MA). Longitudinal sections of the rhopalia were made with the rhopalia embedded on their sides, so that the cells of the touch-plate region, which is under the rhopalium when viewed from the oral side, could be photographed length-wise. Rhopalia from five flight ephyrae and 7 controls were viewed with the TEM and at least two hundred micrographs of the rhopalia were studied.

The IML-2 experiment

Polyps from groups of cultures which had been maintained at 19°C were mixed and rinsed 10 times before they were selected for the experiment. Six polyps of medium size which can produce only two ephyrae were placed in 4 ml of 10^{-5} M iodine in ASW in specially designed cuvettes which permit fixation in space. No air bubbles were permitted. All groups were maintained at 22°C in the Biorack; two groups of polyps were maintained in Type 1 boxes in microgravity and two were on the 1 *g* centrifuge. Four 1 *g* control groups were maintained on the ground. After 12 MET (mission elapsed time) days, one sample from each group was fixed with glutaraldehyde in cacodylate buffer in space. The solutions were injected into the cuvettes after inserting a specially designed bag to collect the effluent solution. The final concentration of glutaraldehyde was approximately 3%. The fixed samples were stored in the refrigerator for two days. Post-flight, the samples were further fixed with fresh glutaraldehyde, rinsed with cacodylate buffer and fixed with osmium for 1 hour (TEM) or 5 minutes (SEM). They were prepared for the SEM and TEM studies as described above. One group of ephyrae were exposed to tannic acid (1%) in cacodylate buffer for 30 minutes after treatment with 1% osmium for 1 hour at 4°C.

Ground control groups were similarly injected with fixative at comparable time periods and were further prepared for the SEM and TEM studies simultaneously with the flight groups. Longitudinal sections of rhopalia were cut as described above. Rhopalia from six ephyrae from the three groups, micro-*g*, 1 *g* in space, and ground-controls, were examined and photographed. A total of more than 600 micrographs of hair cells and statocysts were studied.

In addition to the flight-associated groups, control ephyrae released 96 hours from the strobila were prepared using our standard laboratory method. They were fixed in 6% glutaraldehyde for 1.5 hours, stored in buffer a few days, and post-fixed with osmium for 1 hour (TEM) or 0 or 5 minutes (SEM).

Results

Basic morphology of the hair cells of the touch-plate of ephyrae

An organized touch-plate is not found in newly-released ephyrae until 48 hours after release from the strobila. During the jellyfish-in-space experiments, numerous ephyrae formed in space whether their "parent" polyps were induced to metamorphose with iodine pre-flight or in-flight (Spangenberg *et al.*, 1994a, 1996). Because polyps segment sequentially from the oral to aboral region, ephyrae are released sequentially every 24 hours at 28°C. Those ephyrae induced to strobilate in-flight had been released at different times representing 48-96 hours post-strobilation. Those ephyrae induced 24 hours pre-flight varied between 72-120 hours past their release from their strobilae (having had an extra day for development). Electron micrographs taken of the rhopalia of ephyrae revealed the following information:

Touch-plate surface morphology of controls

Examination of ephyrae 72-120 hours following their release from their strobilae using the SEM revealed hair cells with kinocilia which are surrounded by a group of mv/st (hair bundles) of stacking lengths, the tallest of which are closest to the kinocilium (Fig. 1a). At least 20 mv/st are present per hair bundle (Spangenberg, 1994b) and hair bundles range in size from 1-2.7 μm in width at their bases and from 1-2.5 μm in height. In some cells, lateral filaments are seen between the mv/st (Fig. 2a). The touch-plate hair cells are easily distinguished from other MR cells because their kinocilia are longer, closely grouped, and tend to be straighter. Hair cell kinocilia range in length in these developing touch-plates from 8-18 μm with an average of 12.6 μm. A fine filamentous glycocalyx is seen covering the hair bundle and the base of the kinocilium (Fig. 1b). This material is best seen in samples prepared without osmium treatment. Other kinocilia on the rhopalium are motile and are associated with MR cells both on the touch-plate and in non-touch-plate areas. These kinocilia, which range from 8-12 μm in length (Spangenberg, 1991), are surrounded by a circle of short microvillae of nearly equal length (Chapman, 1974). Fine filaments are often found between the tips of the microvillae and the kinocilium in these MR cells (Fig. 2b). Lateral fine filaments between the microvillae are also found.

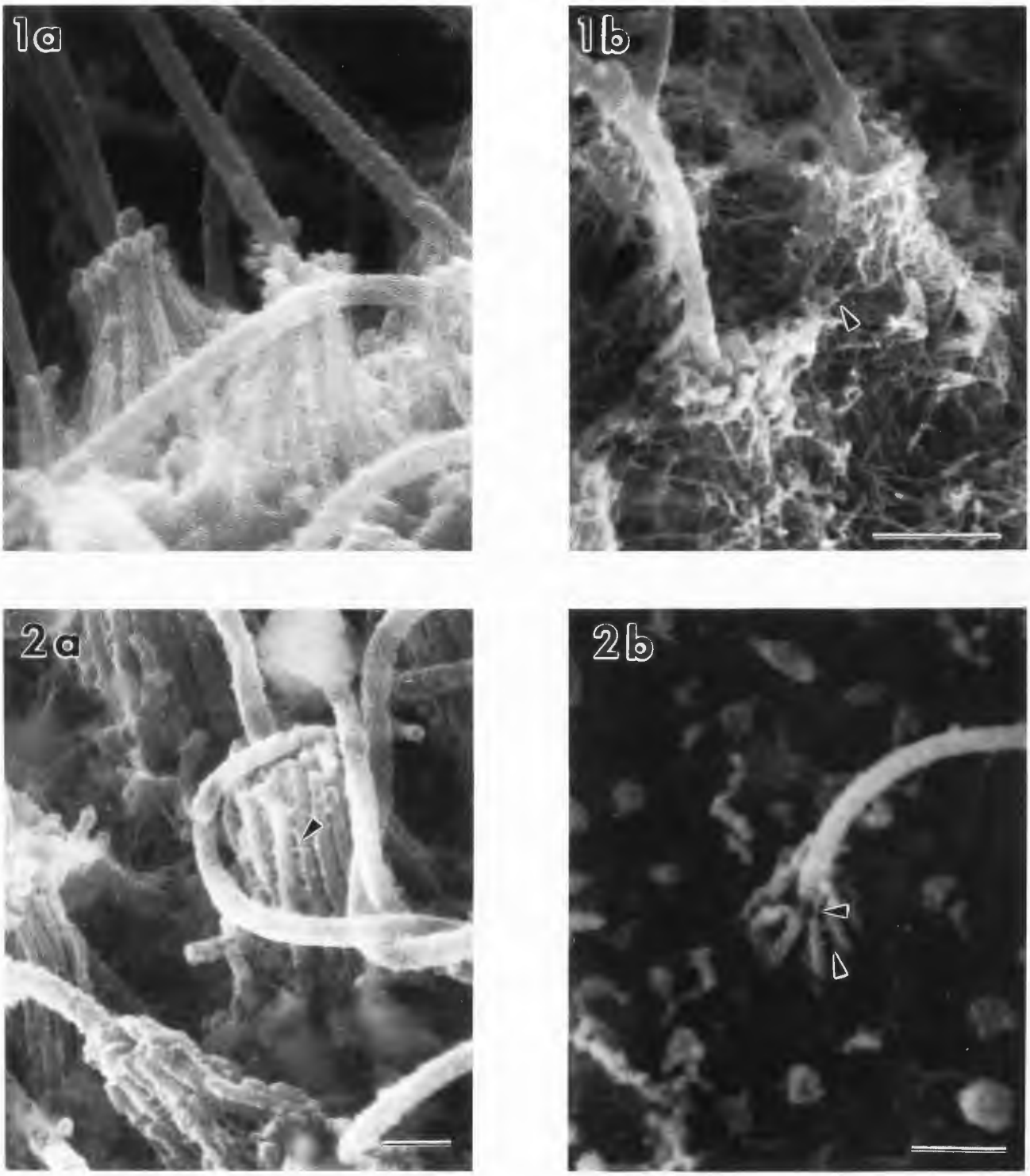
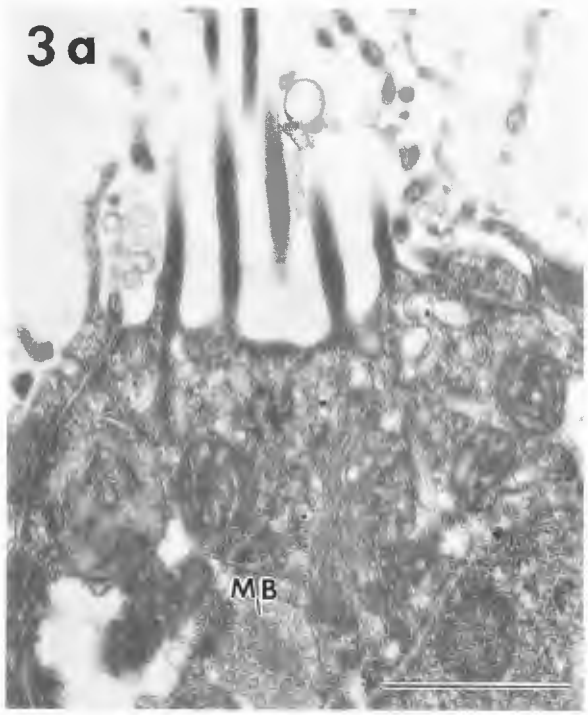
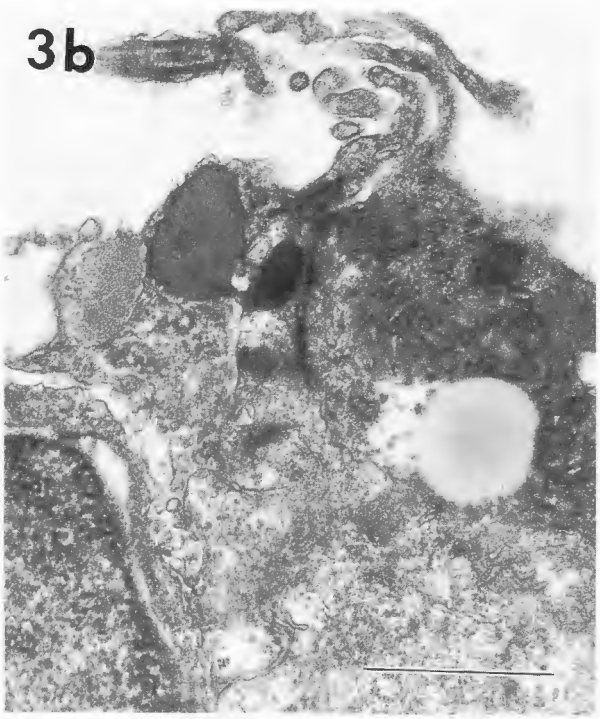
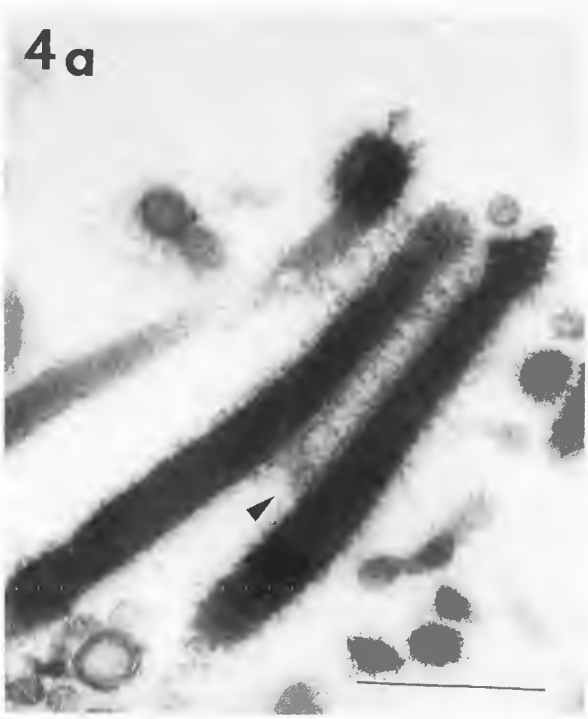


Figure 1. (**a**) Hair cells with mv/st of stairstep lengths in the touch-plate; (**b**) hair cells covered with glycocalyx. Bars = 1 μm.

Figure 2. (**a**) Tiny filaments (arrows) connect sides of mv/st of hair cells; (**b**) tiny filaments (arrows) are seen between mv laterally and between mv and kinocilium of MR cell. Bars = 1 μm.







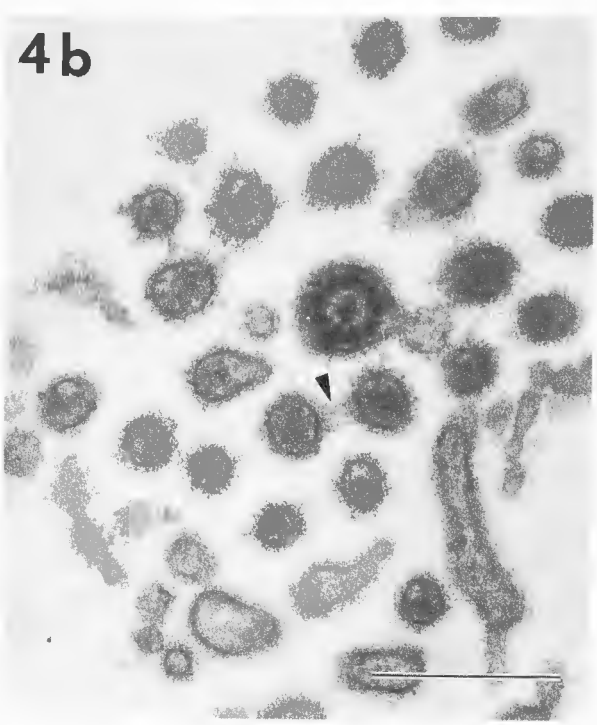
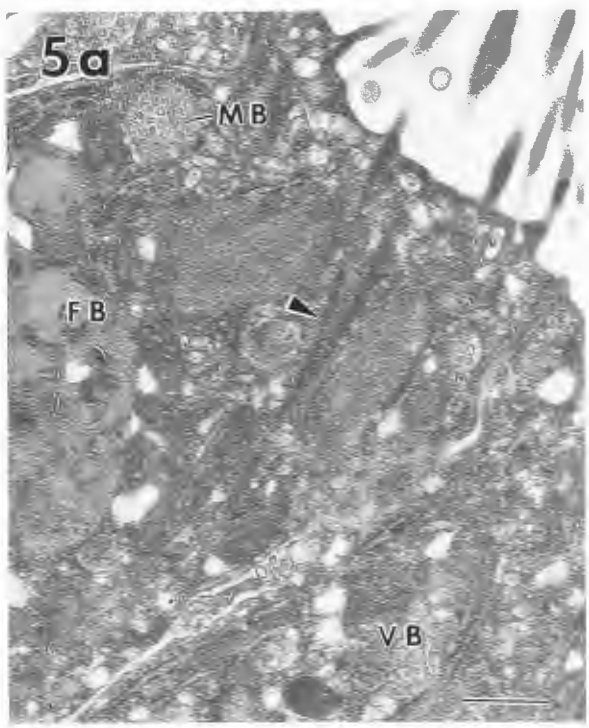
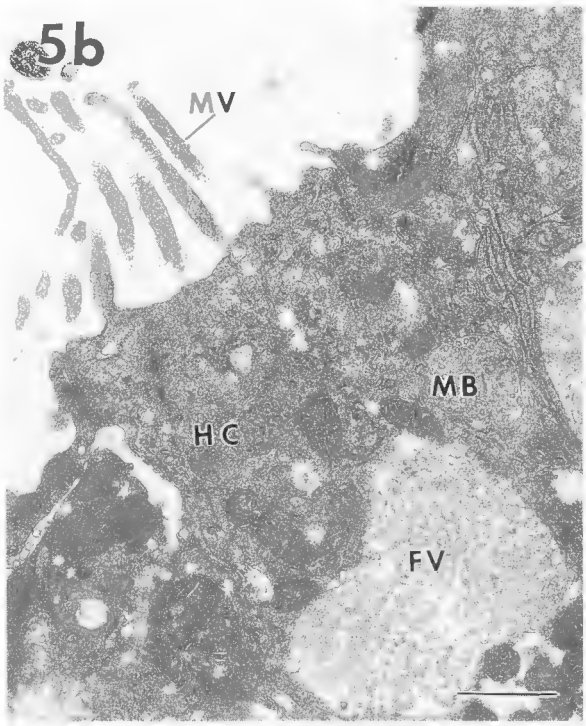
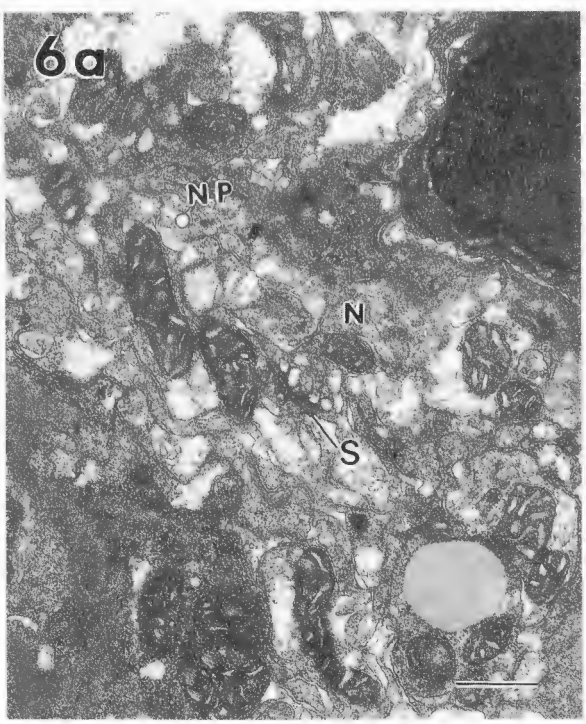


Figure 3. (a) At the TEM level, mv/st often are seen extending from the hair cell to form a pyramid shape; (b) Mv/st bend in association with a bent kinocilium. Bars = 1 μm.

Figure 4. (a) At the TEM level, glycocalyx material (arrow) is seen between the mv/st sectioned longitudinally; (b) glycocalyx material (arrow) between mv/st and kinocilium in cross-section. Bars = 0.5 μm.







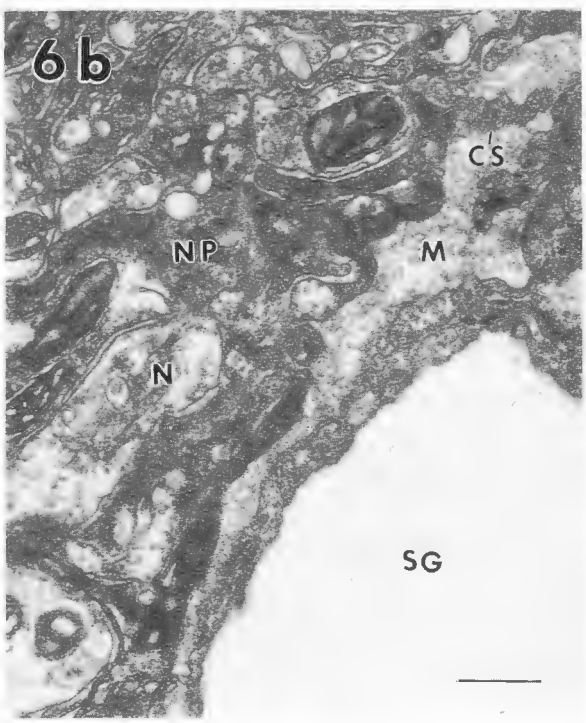


Figure 5. Dense filaments (arrow) extend from the stereocilia into the cytoplasm of the hair cells (**a**, **b**). (**a**) note intracellular multivesicular bodies (MB), fibrous bodies (FB) and vermiform bodies (VB); (**b**) large vacuole with filamentous interior (FV) is seen in the SLS-1 flight ephyra's hair cell. Bars = 1 μm.

Figure 6. (**a**) Neurite plexus (NP) of space-developed ephyra illustrating neurites (N) and a synapse (S); (**b**) neurite plexus region of space-developed ephyra illustrating a cytoplasmic strand (CS) extending across the mesogloea (M) from the neurite plexus of the touch-plate to the statocyst. Note statolith "ghost" (SG). Bars = 1 μm.

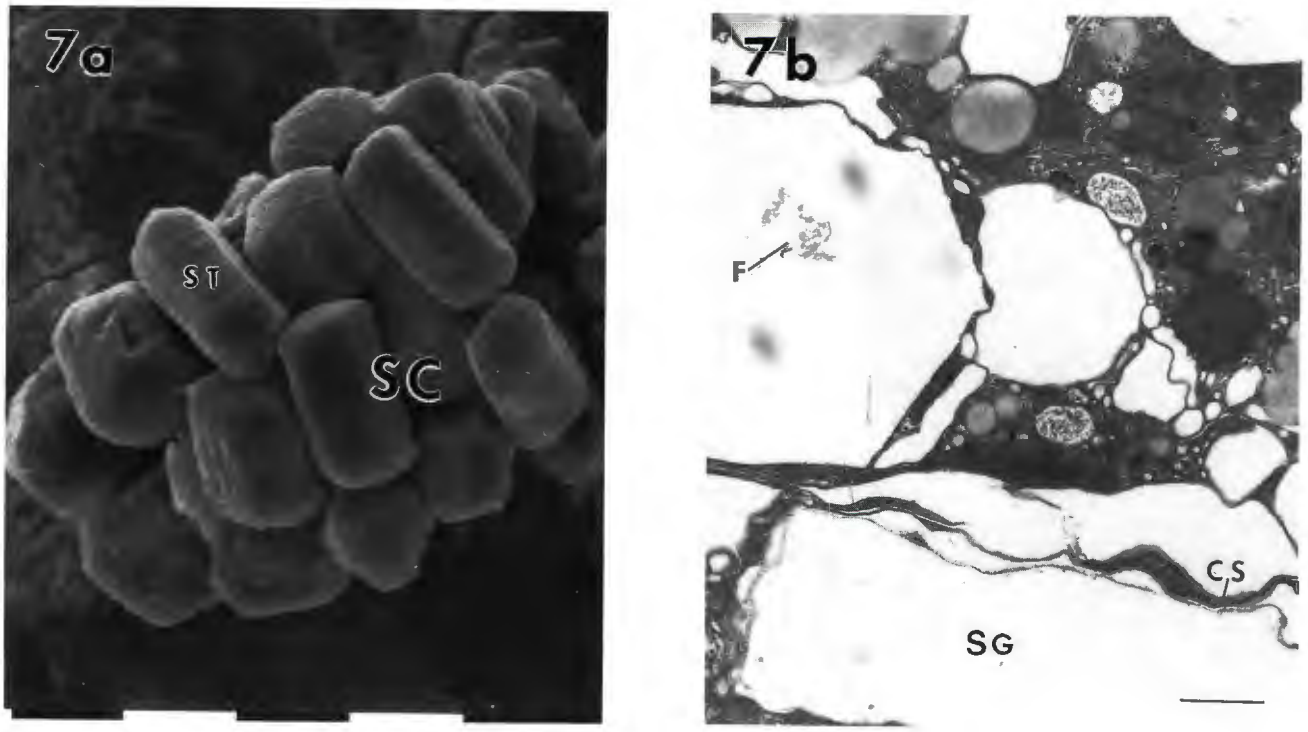


Figure 7. (a) Scanning electron micrograph of Statocyst (SC) with statoliths (ST) of space-developed ephyra. Bar = 10 μm; (b) cytoplasmic strands (CS) of lithocytes which extend between statoliths form a net-work. Cytoplasmic strands from this network and from the neurite plexus are in close contact in the mesoglea. Statolith "ghosts" (SG) show position of statoliths before they dissolved in fixative as well as filamentous material (F). Bar = 1 μm.

Touch-plate ultrastructure

Examination of the developing touch-plate region with the TEM revealed an average of four hair cells per ultrathin section. These cells, which are usually very elongated, have several distinguishing features. At their apex, relatively long mv/st are seen usually extending straight from the cell surface to form a pyramid configuration, but on occasion, bent in coordination with a bent kinocilium (Figs. 3a and 3b). Glycocalyx material can be found connecting microvilli/stereocilia to each other and/or to the kinocilium (Figs. 4a and 4b). The glycocalyx material is especially visible in tannic acid-treated samples. Dense filaments from the stereocilia extend into the cytoplasm of the cells (Figs. 5a and 5b), in some cases extending to the level of the nucleus. In addition, numerous fine filaments are seen in the apex of the cell and at the intercellular junctions, although these cells do not have a cuticular plate as is found in the hair cells of higher organisms. Kinocilial basal bodies are often seen in the cytoplasm but they lack ciliary rootlets. Also at the apex, vacuoles are found in the cytoplasm. Some of these vacuoles have a fibrous (FV) interior and others are empty. Hair cells have multivesicular bodies (MB) (Fig. 5a) with tiny predominantly empty circular vesicles and other bodies with tiny, dense vermiform vesicles. An assortment of inclusion bodies with various amounts of filamentous material (FB) are also found. Whorled figures were rarely found in the hair cells of ephyrae. A prominent Golgi is often seen at the apex of the ephyra hair cells along with groups of mitochondria. Small lipid droplets are also found in the apical region; however, much larger lipid droplets and groups of droplets are found near the base of these cells (Fig. 8a). When few lipid droplets occur in this region, a large space in seen in these cells. Hair cells have a central nucleus which is surrounded by rough endoplasmic reticulum (RER) which is also seen in the apical cytoplasm of the cell. These cells also have a complex collection of neurites (axons) and cytoplasmic strands at their base (Figs. 6a and 6b). Collectively, these neurites and cytoplasmic strands contribute to the neurite plexus, which is found throughout the rhopalium.

Other cells in the touch-plate

Also in the touch-plate area, elongate MR cells with shorter microvillae than are seen in hair cells are found intermingled with the hair cells. A basal body and foot are often seen extending from the kinocilia and a long ciliary rootlet is occasionally found projecting into the

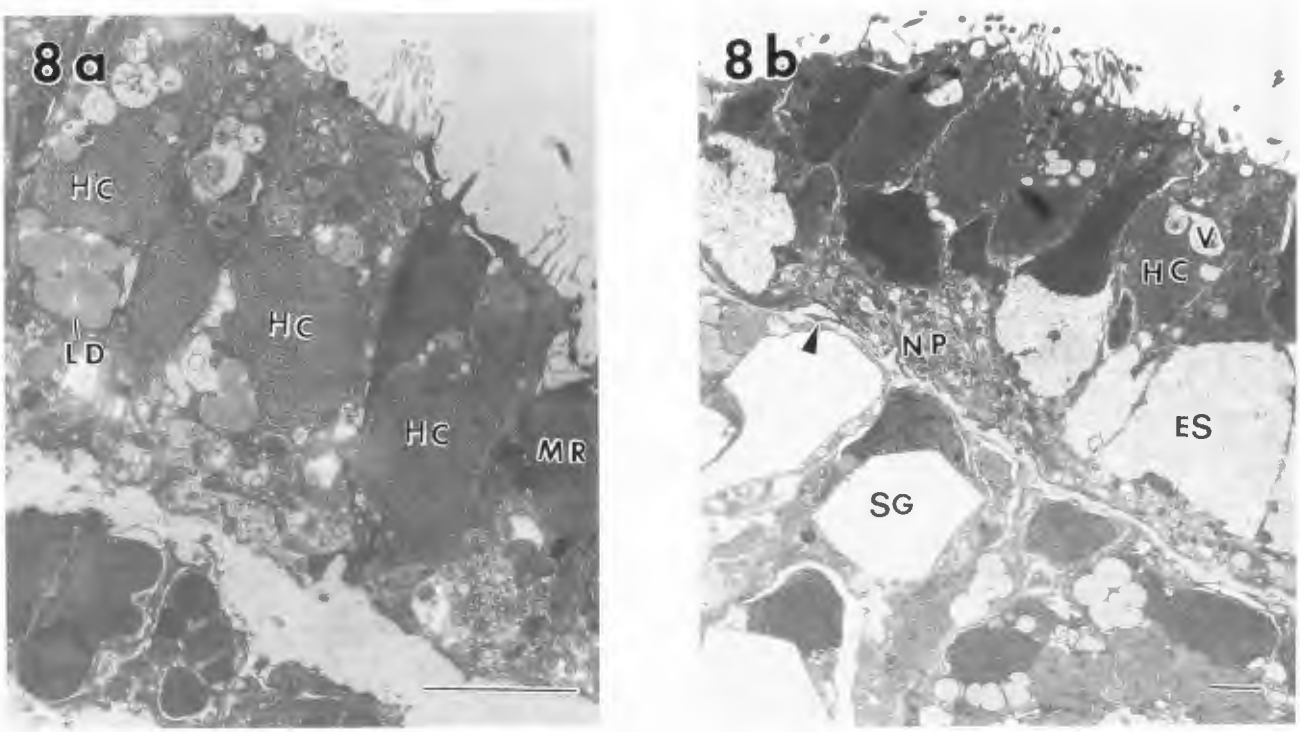


Figure 8. (a) Touch-plate area of rhopalium of Earth-developed ephyra illustrating hair cells (HC) with lipid droplets (LD) at their base and mechanoreceptor cells (MR); (b) touch-plate area of rhopalium of space-developed ephyra illustrating hair cells (HC) with empty spaces (ES) at their bases and the neurite plexus (NP) and the statolith "ghosts" (SG). Bars = 5 μm.

cytoplasm. These elongated cells extend from the surface of the touch-plate to the mesogloea, sending out neurites at their bases. They may be supporting cells as they morphologically resemble the type II cells described by Hundgen and Biela (1982). At the end of the touch-plate distal to the statocyst, another type of MR cell is found. These cells are not as elongate as the hair cells and, although they have kinocilia, their microvillae are much shorter than those of hair cells. These cells have neurites at their base which enter the neurite plexus. Additionally, pigmented ocellar cells of the developing second ocellus are found in the touch-plate region near the tip of the rhopalium. Also, in the touch-plate, neurons send axons into the neurite plexus. MR cells of the rhopalium which are not associated with the touch-plate also send neurites into the neurite plexus. Occasional bipolar synapses (Fig. 6a) are found throughout the neurite plexus.

Touch-plate/statocysts association

Statocysts are well-developed in the 72-120 hours specimens (Fig. 7a). Following preparation for the TEM studies, the statoliths, which are in the cytoplasms of lithocytes, are dissolved by the fixative and remain as crystal "ghosts" (Fig. 7b). These are easily identified as statolith remains because of their size and hexagonal shapes. Within the statolith "ghosts," a filamentous material is seen as described by Spangenberg (1976) previously.

In ephyrae rhopalia, cytoplasmic strands from the statocysts and similar strands from the touch-plate can be seen in the mesogloea (Figs. 8b and 6b). Within the cytoplasmic strands are microtubules and mitochondria (in the wider areas of the strands). Occasionally, these strands from the lithocytes touch those from the touch-plate in the mesoglea, suggesting that these two areas are closely interwoven. In areas where the tissue from the neurite plexus or the epidermis are in close contact with the statocysts but not touching, there is an increase in the filamentous material of the mesogloea. Cytoplasmic strands from the lithocytes also intermingle with similar strands from the ocellar cells of the cup ocellus located beneath the statocyst. Because of the complexity of the neurite plexus area, it was not possible to trace the cytoplasmic strands and neurites from specific cells within the ultrathin sections of the touch-plate.

Comparison of the hair cells and statocysts of ground-based and flight-developed ephyrae

During the SLS-1 and IML-2 jellyfish-in-space

Table 1. Features of the Hair Cells and/or MR Cells of *Aurelia* Ephyrae and medusae and of *Aglantha*.

	Ephyra HC	Ephyra MR	MR I	MR II	MR III	*Aglantha* C cells
1 kinocilium	+	+	+	+	+	+
kinocilium motility	slight	+	NR	NR	NR	-*
number of mv/st	> 20	12	10 - 15	10 - 15	10 - 15	7**
stairstep mv/st	+	-	NR	NR	NR	+
stereocilia with rootlets	+	-	-	-	+	NR
basal body with foot	-	+	NR	NR	NR	NR
ciliary rootlet	-	+	+	+	+	-**
apical vacuoles with fibrous material	+	+	+	+	+	NR
multivesicular bodies	+	-	NR	NR	NR	NR
whorled figures	few	+	+	NR	+	NR
apical Golgi	+	+	+	+	+	NR
large spaces with lipid	+	+	-	-	-	NR
myofibrils at base	-	-	-	+	-	NR
neurites (axons)	+	+	+	+	+	+

+: structure is present -: structure is absent

NR = not reported; Mv/st = microvillae/stereocilia

*From Arkett and Mackie (1988); **From Singla (1983)

MR I - MR III: Medusa data from Hundgen and Biela (1982); MR = Mechanoreceptor cell

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experiments, numerous ephyrae formed in space whether their "parent" polyps were induced to metamorphose with iodine pre-flight or in-flight (Spangenberg *et al.*, 1994a). Those ephyrae induced to strobilate in-flight during the SLS-1 experiment were approximately 48-96 hours past their release from their strobilae and those flown during the IML-2 experiment were 96-120 hours past their release. In addition to the microgravity-exposed ephyrae from space, during the IML-2 experiment, a group of ephyrae developed at 1 *g* through centrifugation in space. Examination of all the groups with the TEM revealed that the rhopalia of the flight-developed and ground control ephyrae were well-developed. The SLS-1 ephyrae which had developed at a higher temperature over a short time period as compared with the IML-2 animals had more vacuoles at their apex with filamentous material (Fig. 5b) inside than did their ground controls. This difference however, was not seen between the IML-2 space-developed ephyrae and their controls. Both the IML-2 and the SLS-1 flight ephyrae had large spaces (Fig. 8b) at the base of the hair cells just below the nuclei with fewer lipid droplets than ground controls (Fig. 8a). Hair cells from all the groups had multivesicular bodies of different sizes as well as various other filamentous bodies apically (Figs. 3a and 5a). Their number and content did not appear to be different between the flight organisms and controls.

Statocysts were well-developed in both the space-developed ephyrae and ground-based controls. No morphological differences were observed between the statocysts of the flight and ground-based ephyrae. Cytoplasmic strands from the statocysts as well as those from the

touch-plate could be seen extending into the mesoglea and occasionally touching in both the flight and control groups.

Discussion

A comparison of the ultrastructural morphology of hair cells of ephyrae 72-120 hours past release from their strobilae with MR cells of rhopalia and with reported accounts of hair cells and MR cells of medusae is presented in Table 1. In addition, the ultrastructure of hair cells and statocysts is compared between ephyrae which developed in microgravity and their controls.

Comparison of ephyra hair cells with other MR cells of the rhopalium

As can be seen in Table 1, hair cells and MR cells of ephyrae have many features in common. The most notable differences are found in the hair bundle associated with the kinocilia. Elongate hair cells with varying numbers of microvillae and stereocilia surrounding a kinocilium in stairstep lengths (Figs. 1a and 1b) found in the touch-plates of ephyrae are not seen in MR cells which have microvillae of nearly equal size but do not have stereocilia. At the ultrastructural level, a long ciliary rootlet is found in the MR cells extending from the kinocilium whereas the hair cells lack this feature. Hair cells, but not MR cells, have stereocilia rootlets extending from the stereocilia into the cytoplasm. Kinocilia of both type cells have basal bodies but a basal body foot was seen only in the MR cells. Whereas whorled bodies are often found in MR cells, they are rarely found in hair cells. Multivesicular bodies, not seen in MR cells, are usually seen in hair cells. These bodies may be a distinguishing feature of the hair cells as they are found in hair cells of higher organisms (Barber, 1968; Anniko, 1983). In ephyrae, both hair cells and MR cells have large spaces at their bases with lipid droplets and neurites extending into the neurite plexus. This feature, apparently, is not found in hair cells of other organisms.

Earlier, Spangenberg (1991) described MR cells in the rhopalia of newly-released ephyrae. She reported kinocilia differences between developing rhopalia of ephyrae during strobilation and newly-released ephyrae. Longer (17-22 μm) polyp-type kinocilia decreased in number as ephyrae developed and shorter kinocilia (8-12 μm) representing MR cells appeared. An organized touch-plate developed within 48 hours after ephyra release from the strobila. It is possible that some of the MR cells on the rhopalia are supporting cells which will differentiate ultimately into hair cells. Anniko (1986) studied the cytodifferentiation of cochlear hair cells in the mouse and determined that cytodifferentiation apparently occurs in a gradient from the hair cell surface to the base. In the developing hair cells, a filamentous substructure in microvillae and stereocilia is seen even before the rootlets of stereocilia are found. The variation in the numbers of stereocilia and microvillae found on the same hair cell and different hair cells of ephyrae may reflect differences in the differentiation of the hair cells in the developing touch-plates.

Comparison of ephyra hair cells with MR cells of medusae

Hundgen and Biela (1982) and Chapman (1974) described MR cells of the touch-plates of medusae of *Aurelia*. Hundgen and Biela found three types of MR cells which they termed types I, II, and III. The medusa MR cells reportedly have microvillae surrounding their kinocilia and these are pictured as being of equal length. Type III cells resemble hair cells in that they have stereocilia with fibrous rootlets which extend into the cytoplasm as do ephyra hair cells. However, they are different from ephyra hair cells in that they lack large basal spaces with lipid droplets which are in ephyra hair cells. Further, medusa MR cells do not appear to have multivesicular bodies, vermiform bodies, or fibrous bodies which are found in nearly all ephyra hair cells. Whorled figures such as those reported in medusa MR cells were rarely found in ephyra hair cells although they were found in MR cells in the rhopalia of ephyrae (Spangenberg, 1991). Elongate MR cells of ephyrae resemble medusa MR cells, especially type II cells although they lack myofilaments at their bases which were found in medusa cells. Another MR cell type which is not elongate and has microvillae is seen in the ephyra's touch-plate, distal to the statocyst. These cells which may be supporting or immature hair cells also send neurites into the neurite plexus. Other cell types found in the ephyra touch-plate region (pigmented ocellar cells and neurons) were not described as being present in the medusa touch-plate region by Hundgen and Biela (1982).

Comparison of ephyra hair cells with those of *Aglantha digitale*

The hair cells of *Aglantha* were studied by Singla (1983) and Arkett and Mackie (1988). They are quite different from those of *Aurelia* ephyrae but some similarities exist (Table 1). Hair cells from *Aglantha* have microvillae of stair-step lengths surrounding their kinocilium as ephyra microvillae/stereocilia surround their kinocilium. *Aglantha* hair cells have fewer microvillae/stereocilia than ephyrae and they extend from a collar which ephyrae lack. The microvilli of *Aglantha* hair cells are 2-3 μm tall and 0.18 μm in thickness and apparently do not have fibrous rootlets which extend into the cytoplasm. In both organisms, the kinocilia are not active. *Aglantha* hair cells also apparently do not have large basal spaces with lipid droplets within. The hair

cells of both animals terminate as a neurite or axon at their bases.

Integration of the cells of the rhopalia

The hair cells and MR cells of the touch-plate are integrated with other MR cells of the rhopalium in the neurite plexus, where non-polar synapses are seen. In addition, our finding that cytoplasmic strands from the neurite plexus of the touch-plate reach across the mesogloea to touch similar strands from the lithocytes of the statocysts suggests a network between these structures which may have functional implications. Movement of the pendulum-style rhopalia during pulsing and swimming could produce stimuli (through stretching or touching) in one part of the structure, e.g., the statocyst or touch-plate which would be rapidly integrated throughout the rhopalium through the neurite plexus. Responses to light may likewise be integrated through this net-work since cytoplasmic strands which are interwoven between the lithocytes were also found extending to cells of the cup ocellus. Earlier, Yoshida and Yoshino (1980) noted that the mesoglea in the ocellar region of rhopalia of ephyrae of *Aurelia* is extremely thin and that not only processes of ectodermal cells including ciliary sheaths but also those of the endoderm cells often penetrate through the thin mesoglea. Some of these processes make up close contacts which these authors believed may be responsible for inducing ciliary cells to differentiate into sensory cells.

Statocyst/statoliths of space-developed ephyrae

Statocysts and statoliths developed as well in the flight ephyrae of both experiments as in the ground controls. Quantitation of the statoliths of the IML-2 ephyrae which were derived from polyps induced to strobilate 24 hours pre-launch, revealed no significant differences in the numbers of statoliths formed in the ephyrae exposed to microgravity, those at 1 *g* in space, and the ground controls (Spangenberg *et al.*, 1996). Earlier, Spangenberg *et al.* (1994b) reported that statoliths of the space-developed ephyrae (SLS-1) induced to strobilate in microgravity showed a statistically significant increase in the number of statoliths formed per rhopalium but those induced on Earth were not significantly different from controls. The X-ray microanalysis profile of statoliths from both groups from the SLS-1 experiment showed a very similar pattern of high levels of calcium and sulfur as is expected in gypsum statoliths (Spangenberg, 1994a).

Hair cells of space developed ephyrae

TEM studies of hair cells of ephyrae which had developed at 28°C in microgravity during the SLS-1 experiment revealed larger vacuoles with fibrous material in their apical region than were seen in controls. In addition, these cells and comparable ones of ephyrae from the IML-2 experiment had large spaces with few lipid droplets at their bases as compared with lipid droplets in the ground-based controls. Although these spaces were nearly empty in the flight animals, many lipid droplets were found in the thin sections in other cells nearby. This finding suggests that lipid metabolism was modified in the space-developed ephyrae's hair cells. Lipid metabolism was found to be affected by space flight in humans. Adipose tissue is a large component of the body weight loss during flight according to Lane and Rambaut (1993).

Apart from the discovery that the ephyrae which developed in microgravity had fewer lipid droplets in their hair cells, the statocysts and touch-plates were not morphologically different from 1 *g* controls. Indeed, no differences in the cytoplasmic strand net-work were found between the rhopalia of the space-developed ephyrae and their controls. This result agrees with that of Vinnikov *et al.* (1983), who, after reviewing effects of weightlessness on developmental stages of amphibians and fish, concluded that the maculae, cristae, and the otolithic apparatus are formed normally in the embryos of these organisms as compared with controls. Thus, they concluded, the gravity stimulus is apparently not a prerequisite for the formation of the structural organization of the vestibular apparatus at the early stages of ontogenesis. Likewise, the gravity stimulus apparently in not a necessary stimulus for the differentiation of the statocyst and touch-plate of ephyrae rhopalia.

Graviceptor function

Little is known about hair cell function in *Aurelia*. Hair cells in frogs transduce mechanical energy to electrochemical energy through the bending of the apical bundle of stereocilia by lateral shearing forces. According to Hudspeth (1985), when the hair bundle is moved toward its taller edge the membrane becomes more permeable to positively charged ions. Mechanoelectrical transduction reportedly occurs near the stereociliary tips (Lumpkin and Hudspeth, 1995) through tip link activity.

Directional bending of kinocilia and ciliary bundles is seen in ephyra hair cells at the ultrastructural level (Fig. 3b). Bending of the kinocilium results in bending of the hair bundle also. Thus far, there are no electrophysiological data to ascertain whether the hair cells function in the same manner as those of the frog. The hair cells of ephryae are directly exposed to the aquatic environment so that the movement of ephyrae from pulsing *per se* could cause bending of the hair bundles as they move through the sea water. In addition, positional changes of the pendulum-style rhopalium during swimming and while sinking probably result in bending of the kinocilia and stereocilia of hair cells when the touch-

plate touches another part of the ephyra's body. Lateral tiny connections are seen between the mv/st of the hair cells, but tip connections between the mv and kinocilia have been found thus far only in the MR cells of ephyrae. It is not known whether these connections function as tip links do in the frog. In addition, ephyra hair cells are coated with a glycocalyx material which could aid in the movement of the mv/st and kinocilium as a unit. If the hair cells of ephyrae function like those of higher organisms, selective bending of the kinocilium and the ciliary bundles would result in the transduction of mechanical information into electrical stimuli which, when transmitted to the neuromuscular-muscular system, could result in directional control during swimming. Failure of the kinocilium and the ciliary bundles to bend in a coordinated manner could possibly result in abnormal swimming behavior.

Ephyrae in microgravity showed more looping and circling behavior than ephyrae on Earth whether they were launched into space as ephyrae or had metamorphosed into ephyrae in space. There were no significant morphological differences seen between the hair cells and statocysts of graviceptors in the space-developed animals as compared with controls which could explain the differences in behavior of the animals in microgravity.

Statocysts of ephyrae in microgravity had as many statoliths as 1 *g* controls, but those in microgravity would have been weightless. Since one explanation for statocyst function involves the "falling of the rhopalial ends weighted with statoliths against the sensory epithelium of the sensory niche (which) seems to constitute the stimulus for the (righting) reflex", (Hyman, 1940), it is reasonable to assume that rhopalia with weightless statoliths in microgravity would not provide the same stimulus as those on Earth. Ephyrae which were pulsing but not receiving adequate positional information from the rhopalia might then be inclined to swim in circles and loops as they did in microgravity. Electrophysiological studies are needed to determine how the network between the touch-plate, statocyst, and neurons of the rhopalia is integrated functionally and to determine the means by which the eight graviceptors interact to control swimming behavior on Earth as well as in microgravity.

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Discussion with Reviewers

R.J. Kingsley: The material in the vacuoles of Figure 5b looks quite vesicular, not at all unlike that of multivesicular bodies. What is the possible relationship between the vacuoles of Figure 5b and the multivesicular bodies? What is the function of the multivesicular bodies?
Authors: We have labelled the bodies which have predominantly small empty vesicles "multivesicular bodies" based on diagrams of them shown in hair cells of higher animals. We do not know the function of multivesicular bodies in the ephyra hair cells and, although they are reported in hair cells of higher organisms, we have not found a reported function for them. The ephyra hair cells appear to have more bodies of various types than are found in descriptions of hair cells of higher organisms. It is possible that some of these types of bodies may be the same types in different stages of development. Further studies of these bodies in hair cells of different stages of differentiation are needed.

A.L. Boskey: Are the differences in lipid distribution in these animals metabolic or structural?
Authors: We believe that the lipid in ephyrae, including the hair cells, is residual lipid from lipid stored in polyps prior to metamorphosis. (The polyps are routinely fed newly-hatched brine shrimp but they received no nutrition during metamorphosis). There is very little information available concerning lipid metabolism in the jellyfish larval forms in general, but we would expect that some of the stored lipid would be utilized by the organisms during metamorphosis. Differences in the lipid of hair cells of flight and ground controls could reflect a difference in ephyra activity between in-flight and ground controls. If so, total lipid content of the flight animals may be different from controls; this will be have to be quantitated in a future flight experiment.

A.L. Boskey: Were any differences in ionic composition or distribution detected during the early time points?
Authors: We only compared the X-ray microanalysis profiles (Spangenberg *et al.*, 1994b) of the statoliths of flight-developed ephyrae induced to strobilate in-flight (SLS-1) and ground controls; these were not different.

M.L. Wiederhold: Is there any indication that there is a significant difference between space-reared and control specimens? If so, what might this difference mean?
Authors: We found only the one difference between space-developed ephyrae and controls: the space-developed hair cells have large empty spaces at their bases where fat globules (droplets) are seen in controls. This result, as shown in the text, was seen in both SLS-1 and IML-2 flown ephyrae. Differences between lipid drop-

lets in flight and ground-based ephyrae may reflect differences in pulsing/swimming activity between these organisms but this needs to be studied further.

M.L. Wiederhold: The term "statocyst" usually refers to an organ that forms a cyst or closed space. Here the term appears to be used to mean the collection of "statoliths" or statoconia. In text, the authors state: "No morphological differences were observed between the statocysts of the flight and ground-based ephyrae". What was measured? How many were measured?

Authors: Statocyst is the term given for the sack of statoliths found at the tip of the rhopalia of scyphozoan jellyfish. The term "graviceptor" is sometimes used to refer to the rhopalium. The statement quoted above is based on observations of the numerous micrographs of statocysts of ephyrae from the SLS-1 and IML-2 experiments. Quantitation of statoliths/rhopalia from both experiments was done post-flight using the light microscope. These data, from statoliths counted in ephyrae post-flight (Spangenberg *et al.*, 1996), revealed that there were no statistically significant differences in numbers of statoliths/rhopalia except in those groups of ephyrae derived from polyps induced to strobilate in-flight during the SLS-1 experiment. Only these ephyrae formed statistically significant higher numbers of statoliths. Note that all of the IML-2 flight animals were induced to strobilate 24 hours pre-flight and the other SLS-1 flight animals were induced 24 and 48 hours pre-flight. All of these groups had statolith numbers which were not significantly different from their controls.

M.L. Wiederhold: The basis of directional sensitivity in vertebrate hair cells, as well as similar receptors in decapods, arises from the stair-step arrangement of stereocilia getting longer in succeeding rows as they approach the kinocilium. This leads to excitation when links between the tip of one row and the side of the next-longer row of stereocilia is stretched. In the jellyfish, the stereocilia are arranged in a circle around the kinocilium. Regular links between the stereocilia and the kinocilium are not described. How would this affect sensory function?

Authors: The stereocilia of the hair cells have stair-step arrangement of stereocilia (see figures in the manuscript), but the MR cells have the circle of microvillae of nearly equal length around the kinocilium. Although illustrations of cells with lateral connections between the mv/st are included, we have not yet detected filament connections between the mv/st and kinocilia in hair cells. Such tiny filaments are found between the tips of mv and kinocilia in the MR cells of rhopalia. It is not known whether such connections function as tip links reportedly function in vertebrate hair cells.

More recently, we have detected glycocalyx material surrounding the kinocilium and hair bundle which may play a coordinating role between mv/st and kinocilia.