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SACCULAR HAIR CELLS IN THE ADULT BULLFROG

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ABSTRACT

We examined the bullfrog's saccule using
light and scanning electron microscopy. We
found no evidence of a striola. Type A
hair cells were not only distributed pe-
ripherally, but also throughout the central
macula, though far less frequently than the
dominant type D. We distinguished two
primary hair cell types, which corresponded
to the ciliary patterns: type A cilia
are associated with short, conical hair
cells, and type D cilia are associated with
long, cylindrical hair cells. Each displays
at least one subtype, which may represent
developmental precursors. The otolithic
membrane is crisscrossed with tunnels and
topped with statoconia.

INTRODUCTION

Numerous crewmen in previous American and
Russian space flights have experienced
motion sickness upon exposure to conditions
of weightlessness. During these episodes,
the performances of the severely afflicted
crewmen seriously deteriorated. Although
the etiology of motion sickness in space
remains speculative, it is known that the
vestibular apparatus, located within the
inner ear, supplies the sensory input for
eliciting the syndrome. According to
recent interpretations, motion sickness
may be caused by unusual patterns of ves-
tibular stimulation, which results in the
sending of mismatched or conflicting inputs
to the central nervous system. The ves-
tibular apparatus contains two divisions:
the otolithic organs and the semicircular
canal endorgans. The otolithic organs are
believed to exert the primary influences
for provoking motion sickness and the semi-
circular canals the secondary.

The bullfrog is serving as the animal model
for assessing functional activities of the
otolithic and canal endorgans. The exper-
iments are conducted by recording from
nerve branches of the saccule and hori-
zontal canal endorgan under static and
dynamic conditions. The saccule, along with
the smaller utricle, are two separate sense
organs that comprise the otolithic organs;
the horizontal canal is one of the three
semicircular canals. The value of the
nerve recordings, while important in them-
selves, is enhanced when correlated with
the structure of the sensory organs. For
example, the hair cells, the sensory re-
ceptors in the otolithic and canal end-
organs, differ in sensitivity, spatial ori-
entation and temporal responsiveness. By de-
termining the hair cell populations and
distributions within the organ, it becomes
possible to associate patterns of nervous
activity with the stimulatory mode of the
hair cells.

Hillman and Lewis (1971) and Lewis and
Li (1973, 1975) identified types A and D
ciliary patterns on hair cells in the bull-
frog's saccule, based solely upon surface
morphologies. They reported that the
type A, which are present in small numbers,
are distributed primarily on the macular
periphery, whereas type D, which are far
more numerous, fill the central macula. In
the present study we examined the saccule
with regard to: (1) the distribution of
hair cells within the macula; (2) the
correspondence between ciliary patterns
and hair cell types; and (3) the rela-
tionships between both the otolithic mem-
brane and the underlying hair cells and
overlying statoconia. Portions of this
data have been previously presented
(Cohen, et al., 1978).

MATERIALS AND METHODS

Adult bullfrogs (Rana catesbeiana) were anesthetized with tricaine methanesulfonate (MS-222). The labyrinth was approached dorsally. The skulls of young adults, which are largely cartilaginous, were relatively easy to penetrate but those of older adults, which are bony, presented more surgical difficulties. Upon removal, the inner ears were immediately immersed in cold (4 C) 2% glutraldehyde that was buffered with 0.1M phosphate or cacodylate containing 1-2 mM Ca and postfixed in buffered 1% osmium for one hour. Because stronger solutions caused considerable cellular shrinkage, their use was discontinued.

The otolithic membrane was removed most satisfactorily by first mechanically cleaning the surface with forceps and then flushing the saccular surface with a fine stream of fluid forced from a syringe. Mechanical cleaning alone was not successful. At first commercial mouthwashes were employed to clean the macular surfaces because they contain surfactants that might have dislodged the otolithic membrane. However, their use was discontinued when we found that soaking not only damaged the surface structures but also did not facilitate the removal of the otolithic membrane. The macula was identified as a slightly denser area within the central saccule. After postfixation, the macula was clearly visible as a dark spot; nerve fibers coursed beneath. Specimens were dehydrated in a graded series of ethyl alcohols (20, 50, 75, 90, 95, 100%) and then critically-point dried in CO_2, coated with a gold-palladium vapor using an Edwards E12E vacuum coater and examined in an ETEC Auto-scan scanning electron microscope at several accelerating voltages. Original magnifications of micrographs ranged from 500 to 10,000. For light microscopy, specimens were embedded in epoxy (Araldite 502) and sectioned on an ultramicrotome (Sorvall MT-1) equipped with glass knifes. The sections were stained with either toluidine blue (Dawes, 1979) or paraphenylenediamine (Estable-Puig, et al., 1956), and then examined with an Olympus microscope under bright field and phase microscopy.

RESULTS

Fixation. Glutaraldehyde, when used as the sole fixative, did not satisfactorily preserve the integrity of delicate surface structures. As a result, microvilli collapsed and disappeared unless the tissue was first stiffened in osmium tetroxide, after which the microvilli formed a dense carpet.

Ciliary Flexibility. When the macular surface was swept with a fine brush made from human hair in order to remove otolithic debris, the stereocilia snapped off at their necks, but the kinocilia generally remained intact. The differences in flexibility between the two ciliary forms are attributable to their proteinaceous cores. The stereocilia contain longitudinal arrays of actin filaments, whereas the kinocilia are filled with paraxially arranged microtubules.

Hair Cells. The macula (sensory epithelium) lies in a shallow basin that is encircled by a slightly elevated rim. There was no evidence of a striola or any topographic contours that are characteristic of its mammalian counterpart. The hair cells are flanked by supporting cells. The two cell types are sealed together at their apical ends by tight junctions. Lewis and Li (1975) identified two distinctive kino- and stereociliary patterns in the bullfrog's saccule, based upon surface morphologies, findings which we have largely confirmed (Cohen, et al., 1978). However, we noted that type A cilia were not only distributed in the macular perimeter but also mingled throughout the central macula, albeit far less frequently than the dominant type D. The type A displays relatively short stereocilia and a kinocilium that is about three-fold longer (Fig. 1). The subtype exhibit slightly longer stereocilia; the subtype exhibits a kinocilium that is taller than the stereocilia. Kinocilia from both types A and D have large bulbous terminals at their apical ends (Fig. 1). However, the bulbous terminals of type A hair cells are usually torn off during the removal of the otolithic membrane. Their bulbous terminals are unusually susceptible because they tower above the smaller stereocilia and therefore lack the protection afforded the type D, in which the shorter kinocilium is tucked in at the side of the domed ciliary bundle.

Upon examining semithin (1μ) sections, we distinguished two primary hair cell types; the short, conical type, and the long, cylindrical type. Each displays subtypes which in the adult bullfrog may represent developmental precursors. In order to combine the findings from light and scanning electron microscopy, the macular surface gently tugged with forceps to separate cells along their seams and expose a lateral face. When viewed with SEM, the conical and cylindrical cell types, corresponded to types A and D ciliary patterns, respectively (Fig. 2). Nonetheless, three factors complicate this assignment. First,

hair cells located at macular periphery are smaller than their counterparts in the central macula. Second, the hair cell bodies at opposite sides of the periphery tilt sharply and centripetally, but acquire a vertical alignment a short distance away. However, the ciliary orientation seem unaffected by the tilt of the hair cell body. Third, kino- and stereociliary heights change from the periphery to the central macula.

Otolithic Membrane. The otolithic membrane is composed of two major components, the gelatinous layer and the statoconia. The gelatinous layer covers the macula in the living frog. However, it is very difficult to preserve the original dimensions and spatial relationships of the gelatinous layer because of its severe shrinkage. As a result, the gelatinous layer of preserved specimens often loses its precise alignment with the underlying ciliary bundles. The shrinkage is more severe peripherally than centrally. In addition, as the layer lifts up, it tears and stretches the thin veiled meshwork that connected the lumenal surfaces of the supporting cells to the gelatinous layer. The gelatinous layer is crisscrossed with anastomosing tunnels. The undersurface formed domes over the ciliary bundles and often enlarged in bulbous chambers. When viewed from beneath or from above in partially cleaned specimens, the gelatinous layer vaguely resembled a honeycomb but clearly lacked its geometric symmetry.

Statoconia. Saccular statoconia vary in size and shape depending upon their distribution within the otolithic membrane. Because most of the otolithic membrane was removed in order to expose the underlying hair cells, the normal statoconial arrangement was lost: the largest statoconia lie on the bottom and the smallest on top. Instead, only statoconia of about the same sizes remained (Fig. 3). However, lodged within the gelatinous layer, smaller "internal" statoconia rested immediately above the ciliary dome and filled the upper portions of the tunnel (Fig. 4). These "internal" statoconia did not directly touch the ciliary bundles, which were shielded by thin veils of fibrous material and by wedges of the dense gelatinous layer.

DISCUSSION

Lewis and Li (1973, 1975) identified two ciliary types, the A and D, in saccular hair cells of the bullfrog, based upon surface morphologies. We have confirmed and extended their findings. First, we found the type A hair cells were not only distributed in the macular periphery but also in the central macula, though they were less common than the dominant type D. Second, the type A kinocilia bore bulbous terminals, which were usually lost during preparative procedures. Third, we were able to match ciliary types with hair cell bodies in most instances. For example, the type A cilia were associated with the short, conical hair cells. The morphology of the conical hair cells changed between the periphery and central macula. At the periphery, these cells were centripetally tilted and possessed the longest kinocilia and shortest sterocila (Fig. 1). However, central conical hair cells were larger and vertically aligned. Their kinocilia were shorter and steriocilia were taller, resulting in a resemblance to type D. On the other hand, the type D cilia were associated with tall, cylindrical hair cells. The latter also varied in size and shape. Thus, the peripheral cylindrical hair cells, in addition to their centripetal tilt, were short and bulbous. They gradually lengthened and assumed a more cylindrical shape and vertical alignment as they approached the central macula. However, we are unable to ascribe the lineage of several intermediates which cluster around the cylindrical hair cells, and bear type D cilia. Although they seem to be transitional forms, they also might be unrelated. Fourth, we do not believe that type A are developmental precursors that transform into the dominant type D during macular growth (Lewis and Li, 1973), though the evidence is admittedly suggestive. The fact that conical hair cells occurred throughout the central macula of the adult bullfrog without having undergone major morphological modification indicates that they are neither developmental precursors to type D nor are they developmentally arrested. The ambiguity results from the typing of hair cells based upon surface morphologies alone. Because A and D ciliary morpholgies differ at the periphery but resemble each other in the central macula, they must be matched with their respective hair cell bodies to ensure accurate identification.

The gelatinous layer of the bullfrog's otolithic membrane is thicker and denser than the laboratory mammal's (Lim, 1973, 1979). It also differs by the presence of a network of tunnels crisscrossing through it and extending downward to the hollow domes of its undersurface, thereby providing the opportunity for fluid movement through the gelatinous layer. We do not know the functional significance of this structural relationship. On its undersurface, the

gelatinous layer overlies the hair and sup-
porting cells. The ciliary bundles extend
upward into the hollowed domes. Although
the correspondence is distorted in fixed
specimens because of shrinkage, the cili-
ary bundles fit loosely rather than snugly
into the large hollowed domes. The ciliary
bundles do not seem to touch the ceilings
of the domes. A thin meshwork or veil fills
the space between the ciliary bundles and
their domes. Dohlman (1971) described the
loose anchoring of the cilia by fibrous
strands. Although the sizes and shapes of
the domes are specific for ciliary types,
they do not directly correspond to the
configuration of the ciliary bundles.

The statoconia are essential for proper
functioning of the otolithic organs (Lim,
1973). The bullfrog's saccular stato-
conia vary in size and shape depending upon
their location. The smaller statoconia
lie on top and the larger ones below them.
(Fig. 3). We were intrigued by the pre-
sence of statoconia within the gelati-
nous layer. These smaller, "internal"
statoconia filled the top of the domes
(Fig. 4) but did not appear to touch the
underlying ciliary bundles, which were pro-
tected by the veil and by wedges of the
denser gelatinous layer. The spatial se-
paration of the statoconia from the ciliary
bundles was obvious in sectioned specimens
but was ambiguous when viewed under SEM
because of the introduction of preparative
artefacts.

In short, the analysis of saccular struc-
ture will be combined with neurophysio-
logical analysis to understand its con-
tributions to balance and motion sickness.

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Fig. 1. Different ciliary patterns at macular periphery. Type A (A) at macular margin list its bulbous kinociliary terminal. Type D (D), the most common, lie inside the margin. Intermediate (I) types may be precursors to other types or represent a separate lineage. X3,780.
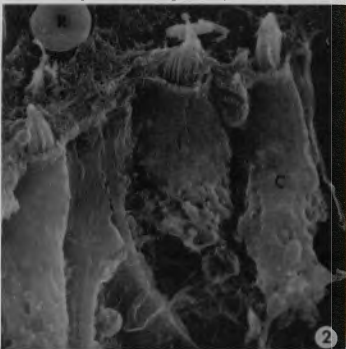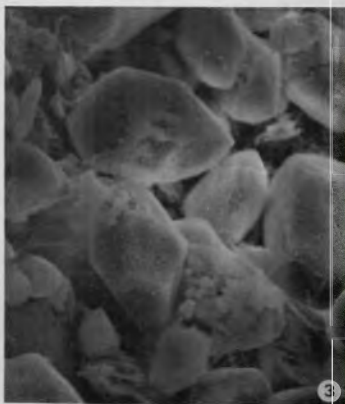

Fig. 3. Statoconia on bottom of mound. The statoconia are pointed at both ends as a result of the sharply angled sides converging. The statoconia vary little in size at this location. Statoconia are smoothly surfaced in fresh preparations but these show etching from fixation. X2,840.

Fig. 2. Lateral view of hair cells in central macular. The long, cylindrical hair cell bodies (C) correspond to the type D ciliary pattern. The short, bulbous hair cells (I) match the intermediate ciliary pattern of Fig. 1. A human red blood cell (R) provides a size perspective. X3,025.
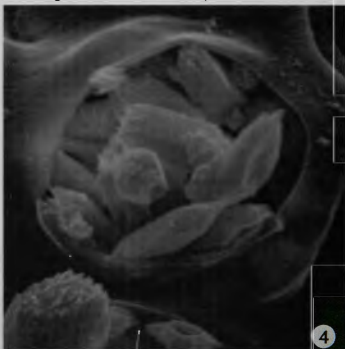

Fig. 4. "Internal" statoconia. These statoconia fill the tunnel that lies directly above the ciliary dome. The internal statoconia are smaller than those comprising the statoconial mount. They are also etched from fixation. X6,620.