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Ultrastructural observations of efferent terminals in the crista Ampullaris of the toadfish, *Opsanus tau*

Published online: 20 May 2004
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Abstract The present study was conducted to visualize the ultrastructural features of vestibular efferent boutons in the oyster toadfish, *Opsanus tau*. The crista ampullaris of the horizontal semicircular canal was processed for and examined by routine transmission electron microscopy. The results demonstrate that such boutons vary in size and shape, and contain a heterogeneous population of lucent vesicles with scattered dense core vesicles. Efferent contacts with hair cells are characterized by local vesicle accumulations in the presynaptic terminal and a sub-synaptic cistern in the postsynaptic region of the hair cell. Serial efferent to hair cell to afferent synaptic arrangements are common, particularly in the central portion of the crista. However, direct contacts between efferent terminals and afferent neurites were not observed in our specimens. The existence of serial synaptic contacts, often with a row of vesicles in the efferent boutons lining the efferent-afferent membrane apposition, suggests that the

efferent influence on the crista may involve both synaptic and nonsynaptic, secretory mechanisms. Further, it is suggested that differences in more subtle aspects of synaptic architecture and/or transmitter and receptor localization and interaction may render the efferent innervation of the peripheral crista less effective in influencing sensory processing.

Keywords Vestibular morphology · Inner ear · Semicircular canal · Nervous system · Balance

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Exp Brain Res (2004) 155:265–273

Due to technical problems, in the printed version, the illustrations were not printed properly. For this reason, the complete article will be reprinted here.

The online version of the original article can be found at <http://dx.doi.org/10.1007/s00221-003-1734-8>

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Introduction

The crista ampullaris of the toadfish horizontal semicircular canal is an inverted saddle-shaped structure which extends across the ampullary enlargement and is anchored at its distal end to the ampullary wall (for a detailed description, see Boyle et al. 1991). Sensory hair cells and supporting cells form a compact receptor sheet that is estimated to be ~940 µm long, and varying in width from ~250–300 µm at the proximal and distal ends, respectively, to ~130 µm at the central isthmus (Boyle et al. 1991). Classically, two types of receptor cells have been distinguished in the cristae of amniotes (reptiles, birds and mammals): flask-shaped type I and cylindrical type II hair cells. Type I cells are surrounded on all but their apical surfaces by specialized enlargements of the primary afferent distal neuritic endings called nerve chalices or calyces (Wersäll 1954; Engström 1960; Hamilton 1968). Type II hair cells contact small bouton endings of primary afferent neurites and the outer faces of neighboring calyces (Spoendlin 1965; Engström 1970; Ross et al. 1985). Anamniotes (fish and amphibians) such as the toadfish

Fig. 1 **A** Schematic of the toadfish horizontal canal crista ampullaris, with the approximate locations of the two sections in **B** and **C** indicated. **B**, **C** One- μm thick survey sections from a resin-embedded toadfish horizontal canal crista, estimated to be $1,008\ \mu\text{m}$ in total length, stained with 1% methylene blue/1% Azur II in 1% Na_2BrO_7 . **B** A section through the peripheral crista, obtained approximately $55\ \mu\text{m}$ proximal to the first appearance of hair cells at the distal margin of the crista. **C** A section obtained approximately $540\ \mu\text{m}$ from the distal margin of the same crista, through the isthmus. The arrows indicate the middle 50% of the width of the crista. Black scale bar: $25\ \mu\text{m}$ for both micrographs

have traditionally been described as having only type II-like elements (Wersäll 1960; Flock 1964; Gleisner et al. 1973). Since the presence of a nerve calyx has often been used as the signal feature to distinguish Type I from Type II hair cells in amniotes, the absence of this calyx in anamniotes has been used to support the classic notion that

only one type of hair cell is present in these animals (Wersäll 1960; Flock 1964). However, there is increasing evidence for the existence of a multiplicity of morphologically defined hair cell types in anamniotes (Popper 2000). Although early ultrastructural studies did indeed demonstrate the existence of morphologic variations in the hair cells of bony fishes (Flock 1964; Lowenstein et al. 1968; Wegner 1982), the demonstration that these might not all be variants of mammalian type II cells is more recent (Saidel and Presson 1990; Saidel et al. 1990; Chang et al. 1992; Lanford and Popper 1996; Saidel and Crowder 1997) and suggests that a revision of the classic scheme is in order. Synapses between hair cells and primary afferents occur at morphologically discrete contacts that are often distinguished by the presence of a synaptic body with a halo of electron-lucent vesicles in the receptor cell, and a postsynaptic density associated with the afferent terminal membrane (Sans and Highstein 1984).

Vestibular end organs receive CNS innervation from several cell groups located in the caudal brainstem (Highstein and Baker 1986) (for review, see Goldberg et al. 2000). In amniotes, these efferent axons terminate both on the nerve calyx, forming classic morphologic synapses with symmetrical membrane thickenings, and directly on type II hair cells. Direct hair cell contacts involve a synaptic complex that includes a flattened subsynaptic cistern subjacent to the hair cell membrane (Smith and Sjostrand 1961; Hama 1965; Nakajima and Wang 1974). Electrical stimulation of the efferent vestibular system (EVS) in toadfish affects the discharge of primary afferents and their responses to head movements (Highstein and Baker 1986; Boyle and Highstein 1990). During EVS activation, the firing rates of acceleration and high gain afferents are increased up to fourfold, while simultaneously the amplitude or sensitivity of their responses to head velocity is scaled downward by about 20%. Low gain afferents show a slight increase in firing rate, on average 25%, and their responses to head velocity are essentially unchanged. It has been suggested that this increase in afferent firing rate may be attributable to direct efferent contacts on afferent endings (Highstein and Baker 1985), whereas the reduction in sensitivity may be due to the hyperpolarizing action of efferent synapses onto hair cells (Russell 1971). In the toadfish, two morphological types of brainstem efferent terminals and contacts have been described previously (Sans and Highstein 1984). Efferent synapses onto hair cells reportedly involve terminals with round synaptic vesicles and electron-dense particles pre-synaptic to hair cell membranes with associated subsynaptic cisterns, whereas efferent terminals with round lucent microvesicles and some dense core vesicles form synapses directly with afferent endings. The present study was conducted to re-examine the synaptology of the efferent endings in the toadfish crista ampullaris. Preliminary findings were reported in abstract form (Holstein and Highstein 1999; Holstein et al. 2000).

Materials and methods

The tissue for this study was obtained from twelve adult oyster toadfish (*Opsanus tau*) of either gender (approximately 500 gm) obtained from the Marine Biological Laboratory (Woods Hole, MA, USA). The experiments were approved by the Institutional Animal Care and Use Committee at the Marine Biological Laboratory and were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23, Rev. 1996).

Toadfish were anesthetized by immersion in MS 222 (25 mg/L sea water, 3-aminobenzoic acid ethyl ester; Sigma, St. Louis, MO, USA), partially immobilized by intramuscular injection of pancuronium bromide (0.05 mg/kg.), and heparinized (1 ml of 500U diluted to 50% in saline) by injection into the conus through a 30G needle. A blunt perfusion needle (18G), attached via silicon tubing to a variable speed peristaltic pump, was inserted into the conus. The vasculature was flushed for 2–3 min with approximately 50 ml of room temperature (RT) cacodylate-buffered saline (0.01 M, pH 7.3) containing 5% sucrose and 2 μ M sodium nitroprusside, and then perfusion continued with injection of 300 ml of RT fixative (4% paraformaldehyde/3% glutaraldehyde) in cacodylate buffer (0.1 M, pH 7.4) delivered at 18 ml/min, followed by 300 ml of the same fixative at 4°C delivered at the same rate.

After completion of the perfusion, a dorsal craniotomy was performed to expose the vestibular labyrinths. The dura was opened and an additional 5 ml of perfusion fixative was injected over the region of the labyrinths and left in situ for 10 min. The semicircular canals were then removed from the skull and placed in vials of glutaraldehyde-free fixative (immersion fixative) for 2 h at 4°C. The ampullae were subsequently dissected from the canals and placed in vials of fresh immersion fixative at 4°C for 12 h. The end organs were then osmicated (1% OsO₄), dehydrated, infiltrated with Epon-Araldite resin, embedded as whole-mounts, and cured at 56°C for 4 days. No en bloc or post-microtomy staining was performed. As a result, the sections have low contrast but are free of staining artifact.

Sections of the resin-embedded tissue were cut by ultramicrotome (Leica UCT) in the transverse plane, perpendicular to the long axis of the crista, and mounted on Formvar-coated slot grids. The following sectioning strategy was utilized: (1) five microns of sensory epithelium were thin-sectioned, which usually required 80–85 sections of 60-nm thickness each; (2) a 1- μ m survey section was cut and then 50 μ m (in peripheral crista) or 40 μ m (in central crista) of tissue were discarded; (3) another 1- μ m survey section was cut, and then another 5 μ m of tissue was thin-sectioned as above. This cutting sequence was repeated until the entire crista was sectioned. The 60-nm section thickness aided resolution, but further decreased contrast in the micrographs. Tissue from the peripheral (Fig. 1A) and central (Fig. 1B) regions of the crista was collected and examined separately. The 1- μ m survey sections were collected on glass slides, stained with 1% methylene blue/1% Azur II in 1% Na₂BrO₇, dehydrated, coverslipped using DPX (BDH Laboratories, Poole, England), examined using a Zeiss Axiophot light microscope, and documented using a Spot (Diagnostic Instruments) digital camera. Thin sections were examined and photographed using a transmission electron microscope (Hitachi H7500). All electron microscope negatives were printed for analysis; those used as illustrations in this manuscript were subsequently digitized using an Agfa Duoscan T2500 scanner. All digital images were prepared as figures using Adobe Photoshop. Before printing, such images were adjusted for brightness, contrast, evenness, and balance across panels in multiple panel figures using only those methods that are analogous to routine wet darkroom techniques.

Results

Cells and endings were identified solely on the basis of ultrastructural criteria. Hair cells in the toadfish crista are distinguished from supporting cells by the presence of a

Fig. 2 A low-magnification electron micrograph of a transverse section through the horizontal canal crista, approximately 56 μ m proximal to the peripheral margin. Three hair cell bodies (HC) and four efferent boutons (numbered 1–4) are apparent in the figure. All four boutons are located deep in the crista, just above the nuclei of the supporting cells. The toadfish hair cells are characterized by the presence of a dense system of filaments that stream in three dimensions through the cytoplasm, particularly in the hypolemmal region (arrows). When cut in cross section, these filaments have the appearance of short tic marks. Filaments can be seen at higher magnification in Figs. 6, 7 and 8. Scale bar: 2.5 μ m

cuticular plate, round nuclei, and ample cytoplasm containing a variety of cellular organelles. Filaments and microtubules of variable length course through the cytoplasm of the hair cell body in three dimensions, particularly in the outer margins of the soma (Fig. 2). These organelles do not appear to be present in mammalian hair cells, but are a prominent feature of the cytoplasm in hair cells of the cristae ampullares in goldfish (Lanford and Popper 1996) and toadfish (Sans and Highstein 1984), and the saccular maculae of goldfish (Hama 1969) and catfish (Jenkins 1979). Somal spines or leaflets often interdigitate between adjacent efferent and afferent endings. As noted by other investigators using similar tissue processing techniques (Hama 1969; Chang et al. 1992; Lysakowski 1996; Lysakowski and Goldberg 1997; Ross 1997), the distinction between efferent and afferent endings is usually straightforward. Profiles of afferent endings have electron-lucent cytoplasm and occasional postsynaptic densities seen in association with hair cell-afferent synaptic body synapses. Efferent terminals have denser cytoplasm and are highly vesiculated with focal accumulations of presynaptic vesicles in the region generally opposite to the subsynaptic cisterns in the

Fig. 3 A paddle-shaped efferent terminal (*e*) adjacent to a hair cell (*hc*) in the peripheral crista. Vesicles are compartmentalized in the interconnecting process of the efferent fiber (*arrows*) and in the bouton head, with mitochondria clustered in the intervening portion of the terminal. Scale bar: 0.7 μ m

hair cells. Although vesicle-containing afferent terminals have been reported to form reciprocal synapses with type II hair cells in other systems (Lysakowski 1996; Lysakowski and Goldberg 1997; Ross 1997), no such contacts were observed in our material. The identity of approximately 1% of the profiles was equivocal; these were excluded from consideration.

Although efferent boutons are present throughout the crista, synaptic contacts involving these terminals are not observed in our material in the most peripheral margins of the epithelium. This comprises <10% of the crista, 4–5% at either longitudinal end. Hair cells in the remaining 91% of the crista receive direct innervation from efferent vestibular fibers. In general, the efferent boutons are found deep in the epithelium (Fig. 2), near the basement membrane. The synaptic contacts are formed primarily with the basal aspect of the hair cells and occasionally the perinuclear zone, but efferent terminals are not observed to form synapses with the supranuclear or apical portions of the receptor cells.

Most of the efferent boutons are of small size. The cross sectional diameters of the endings average $1.83 \pm 0.6 \mu$ m (mean $\pm$ standard deviation) and range from 0.6–3.87 μ m ($N=314$). The terminals contain dark tubular mitochondria with narrow intercrestal spaces and a moderate density of round/pleomorphic lucent synaptic vesicles, as well as occasional scattered dense core vesicles. The mitochondria

Fig. 4A–C Examples of the long, narrow subsynaptic cisterns (*arrows*) characteristic of synapses formed between efferent boutons (*e*) and hair cells (*hc*). **A** A large mushroom-shaped bouton with dense accumulations of round/ovoid synaptic vesicles and scattered dense core vesicles (*arrowheads*) makes two synaptic contacts with the adjacent hair cell. **B, C** Additional examples of long, narrow subsynaptic cisterns postsynaptic to efferent terminals of disparate morphology. The terminal in **B** has a moderate density of large spherical vesicles; the profile in **C** contains a cluster of pleomorphic vesicles at the contact site. Scale bars: 0.5 μ m in **A**; 0.25 μ m in **B, C**

Fig. 5. **A** A cup-shaped efferent bouton (*e*) contacting two hair cells (*hc*) (black and white arrows) in the peripheral crista. Most of the mitochondria in the terminal are concentrated in the concavity of the profile, with vesicles filling the rim. The synaptic region delineated by the rectangle in **A** is shown at higher magnification in **B**, where a narrow subsynaptic cistern and densely packed lucent synaptic vesicles are more clearly visible. The postsynaptic location

of the cistern is incompletely co-extensive with respect to the presynaptic membrane density. The white arrows in **B** indicate the extent of the subsynaptic cistern in the hair cell and the black arrows indicate the extent of the synaptic contact region where there is a local accumulation of vesicles adherent to the presynaptic membrane. Scale bars: 1 μm in **A**, 0.25 μm in **B**

tend to cluster in one region of the profile, away from the synaptic vesicle pool (Figs. 3 and 4).

The majority of efferent boutons are spherical or oval, although barbell-, cup-, paddle- and mushroom-shaped terminals are also present (Figs. 2, 3 and 4). The endings provide multiple inputs to individual hair cells (Fig. 4A) and to two neighboring hair cells in single or adjacent thin sections (Fig. 5).

The synaptic contacts between efferent boutons and hair cells tend to be small in all dimensions, $\sim 100\text{--}150$ nm thick, with a 50-nm wide synaptic cleft. Subsynaptic cisterns are present in the hair cells at regions of synaptic contact (Figs. 4, 5 and 6), although the postsynaptic location of the cistern is often offset or incompletely co-extensive with respect to the presynaptic membrane density (Fig. 5B).

In contrast to the ubiquitous efferent-hair cell synaptic contacts, direct efferent synapses onto afferent processes are less evident in our material. Instead, serial synapses are frequently observed in which a hair cell receives efferent innervation through a cisternal synapse and in turn contacts an afferent neurite at an adjacent synapse (Figs. 6 and 7). These latter contacts are usually characterized by one or more large synaptic bodies surrounded by annuli of lucent vesicles in the presynaptic hair cells. The serial synapses are common in the central crista: a hair cell-afferent synapse is present in the same or an adjacent thin section in approximately 40% of the sample of efferent-hair cell contacts. However, these serial synaptic arrays are present in less than 10% of the sample of efferent-hair cells contacts in the peripheral crista.

At some serial synapses, a row of vesicles can be seen in the efferent terminal at the apposed membranes of the efferent and afferent endings (Fig. 8). Although occasional dense core vesicles were present in efferent terminals in the peripheral crista, they were more common in the

Fig. 6 A serial synaptic contact in the central crista. The efferent terminal (*e*) contacts a hair cell (*hc*) through a subsynaptic cistern (black arrows). The hair cell, in turn, forms a synapse with an afferent neuritic process (*a*) at a nearby junction marked by the presence of synaptic bodies (white arrows). Presynaptic coated vesicles are present at the HC-afferent contact sites. Scale bar: 0.5 μm

central crista in general, and particularly in those efferents which had direct membrane appositions with afferents. However, such contacts comprised less than 10% of the total population of efferent synapses.

Fig. 7 A serial synapse in the central crista. The efferent terminal (*e*) contacts a hair cell with a long, narrow subsynaptic cistern (*black arrows*). The hair cell contacts an afferent neurite (*a*) through two synapses (*white arrows*) characterized by vesicles adherent to, or fused with, the presynaptic membrane and the presence of postsynaptic densities at the contact sites. Scale bar: 0.5 μ m

Discussion

The present study describes the ultrastructural features of efferent boutons in the toadfish horizontal canal crista. These boutons are of variable shape and size, and contain a heterogeneous population of lucent vesicles with scattered dense core vesicles. Efferent contacts with hair cells are characterized by the presence of a local accumulation of vesicles in the presynaptic terminal and a subsynaptic cistern in the postsynaptic region of the hair cell. Serial efferent-hair cell-afferent synaptic arrays are common, particularly in the central portion of the crista. Surprisingly, however, direct contacts between efferent terminals and afferent neurites are not observed in our specimens, although the vesicle accumulations and membrane associations at some efferent-afferent junctions suggest a nonsynaptic, secretory basis for intercellular communication (Verhage et al. 1991; Golding 1994; Leenders et al. 1999).

The efferent pathway has been recognized in a wide variety of mammalian and non-mammalian vertebrates, including rat (Schwarz et al. 1986), guinea pig (Strutz 1982), gerbil (Perachio and Kevetter 1989), cat (Gacek and Lyon 1974), chinchilla (Lysakowski and Goldberg 1997), and squirrel monkey (Goldberg and Fernández 1980), as well as birds (Whitehead and Morest 1981), reptiles (Roberts and Meredith 1992), amphibians (Fritzsch et al. 1984) and fish (Fritzsch et al. 1989), including toadfish (Highstein and Baker 1986), although such a pathway has not been observed in the primitive vertebrate hagfish (Baird 1974a, b). The fibers originate from cells in the medulla which are located between the vestibular and abducens nuclei in mammals and in the vicinity of the facial motor nucleus in non-mammalian vertebrates (for review, see Goldberg et al. 2000). In the toadfish, two groups of efferent cell bodies have been identified: fusiform neurons located dorsal to the medial longitudinal fasciculus and pyramidal neurons situated

Fig. 8 Serial synapse with efferent-afferent membrane apposition in the central crista. The efferent terminal (*e*) contacts a hair cell with a subsynaptic cistern (*black arrows*). The hair cell contacts an afferent neurite (*a*) through an adjacent specialization (*white arrows*). Occasional scattered dense core vesicles are present in the efferent terminals (*black arrowheads*). In addition, a row of such vesicles lines the efferent-afferent membrane apposition (*white arrowheads*). Scale bar: 0.5 μ m

lateral to that tract (Highstein 1991). A similar pair of efferent brainstem nuclei has been reported in frog (Birinyi et al. 2001).

The ultrastructural features of the efferent terminals are remarkably similar among vertebrates (Lysakowski 1996). In general, the boutons contain a variable density of small, clear, round synaptic vesicles together with occasional larger dense core vesicles. The small clear vesicles are likely to contain acetylcholine (ACh), which has been demonstrated to be at least one of the efferent neurotransmitters (for review, see Guth et al. 1998). Similarly, the dense core vesicles are thought to contain neuroactive peptide transmitters or modulators, most likely calcitonin gene-related peptide (Tanaka et al. 1988, 1989; Wackym et al. 1991, 1993), enkephalins (Carpenter et al. 1987; Perachio and Kevetter 1989) and/or substance P (Usami et al. 1991). Thus, there is no clear correlation between these two efferent cell groups and peripheral bouton morphology.

The efferent terminals form synaptic contacts with type II hair cells, and with both calyx and bouton afferent endings in amniotes (Smith and Rasmussen 1968; Iurato et al. 1972; Wersäll and Bagger-Sjöbäck 1974). Although direct efferent innervation of afferent processes has been reported in some fish (Nakajima and Wang 1974; Sans and Highstein 1984; Lanford and Popper 1996; Lysakowski 1996), they have not been observed in frog (Lysakowski 1996) or in the material for the present report. Efferent contacts with hair cells lack pronounced synaptic membrane densities, but are distinguished by a subsynaptic cistern in the hair cell cytoplasm opposite the presynaptic bouton. Such cisterns are not present at efferent synapses onto afferent processes (Iurato et al. 1972). In summary, the fine structure of the efferent boutons in the toadfish is indistinguishable from that of other vertebrates. However, the synaptology of these endings has several unique attributes, most notably the serial synaptic arrays that are

common in the central crista and the vesicle accumulations at serial synaptic efferent-afferent membrane appositions.

In a previous study of the toadfish vestibular periphery (Sans and Highstein 1984), efferent synapses onto hair cells were reported to involve terminals with round synaptic vesicles and electron-dense particles. Other efferent terminals, with smaller round lucent microvesicles and some dense core vesicles, directly contacted afferent endings. These direct efferent-afferent contacts were apparently quite common. These findings differ in several respects from our present observations. Although the vesicle population in the efferent boutons in our material shows some variability in size and marked differences in shape, there is no correlation between vesicle morphology and other anatomical attributes of the endings. In addition, no electron dense particles are present in a particular class of efferent boutons in our study, and no microvesicles are observed. Although these differences could be attributable to alternative tissue fixation or processing approaches, our failure to find direct efferent-afferent contacts is less likely to be due to methodologic issues. Even assuming that some such contacts exist in the toadfish but were overlooked in our study, it is very unlikely that this synaptology comprises a significant synaptic population since our sampling strategy encompassed the entire extent of the epithelium. It is most likely that the disparate results are due to differences in synaptology between sensory organs. In fact, Nakajima and Wang examined tissue from the goldfish saccule, and Sans and Highstein pooled data from the utricular macula and the crista ampullaris. While the latter investigators report that the ultrastructural features were generally similar, it is not possible to determine what impact this tissue pooling may have had on their observations. At present, we interpret the disparities between our present findings and theirs to be due to this tissue pooling, and speculate that direct efferent-afferent contacts are concentrated in the otolith organs of the toadfish. The serial efferent-hair cell-afferent synapses, though not previously reported, are quite prevalent in our material, particularly in the central region of the crista. The existence of these contacts, and the row of vesicles in the efferent boutons lining the efferent – afferent membrane apposition, suggests that the efferent influence on the crista may involve both synaptic and nonsynaptic, secretory mechanisms.

Efferent activation of vestibular hair cells in the toadfish produces large hyperpolarizing postsynaptic potentials, decreases membrane resistance, and decreases the hair cell receptor potential modulation in response to canal stimulation (Boyle et al. 1997). Concomitantly, the afferent firing rate increases, and the gain in response to adequate stimulation decreases (Boyle and Highstein 1990; Boyle et al. 1998). In the toadfish (Highstein and Baker 1985; Boyle and Highstein 1990), as in turtle (Brichta and Goldberg 1996, 2000) and frog (Rossi et al. 1980, 1994; Bernard et al. 1985; Rossi and Martini 1991), afferents innervating the peripheral crista are less sensitive to efferent stimulation than those innervating the central torus region, although the polarity of that sensitivity, excitation

or inhibition, is variable. Similarly, in cat (McCue and Guinan 1994) and chinchilla (Plotnik et al. 2002), afferent discharges associated with processes innervating the central crista appear to be more responsive to efferent stimulation. It is unlikely that the regional differences in efferent response to afferent stimulation are attributable to differences in the efferent innervation density per se (Purcell and Perachio 1997). However, it is very likely that differences in other, more subtle aspects of synaptic architecture and/or transmitter and receptor localization and interaction may underlie this behavior, rendering the efferent innervation of the peripheral crista less effective in influencing sensory processing. The presence of a substantial direct efferent innervation of peripheral hair cells is a further puzzle, given that the responses of low gain afferents to adequate stimulation are only slightly reduced by efferent activation. This suggests that the EVS may serve multiple functions, in addition to its role in reducing the responses to head velocity.

It is conceivable that structural aspects of the subsynaptic cisterns at efferent-hair cell junctions support regional differences in Ca^{2+} concentration (Miller 1991), since longer cisternal pockets may provide greater storage capacity, and therefore also greater local Ca^{2+} buffering capability in the microenvironment of the efferent synapse. In fact, activation of sympathetic ganglion cell cisternal synapses causes Ca^{2+} release from the cisternae, opening Ca^{2+} -activated K^+ channels and thereby hyperpolarizing the cell (Fujimoto et al. 1980). Moreover, application of ACh, a putative efferent neurotransmitter, on isolated cochlear hair cells produces an outward K^+ current and membrane hyperpolarization (Fuchs and Murrow 1992). These findings suggest that Ca^{2+} is released from intracellular stores and causes increased K^+ conductance. In this framework, the efferent vestibular pathway would regulate hair cell threshold and gain by controlling intracellular Ca^{2+} release from the cisternae, and therefore Ca^{2+} -activated K^+ conductance (Shigemoto and Ohmori 1990; Fuchs and Murrow 1992; Yamashita et al. 1993). If so, then differences in cisternal size would predict regional differences in Ca^{2+} storage capabilities, as well as more generally in the regulation of cytosolic free Ca^{2+} concentration.

Another possible role for the subsynaptic cisterns may be purely architectural. It has been noted that Ca^{2+} traverses the hair cell basal plasma membrane through a variety of receptor- and membrane-associated channels (Fuchs 2002). Although ACh also raises the Ca^{2+} concentration in the intracellular cytosolic pool, afferent and efferent synaptic Ca^{2+} signals are probably segregated in the hair cell (Lenzi and Roberts 1994). In this context, the cisterns may serve to maintain this independence by providing a diffusion barrier as well as an intracellular storage compartment (Fuchs 1996; Guth et al. 1998) to both supply and sequester Ca^{2+} (Guth et al. 1998; Rispoli et al. 2001).

Acknowledgements The authors are grateful to Ms. Rosemary Lang for technical help with various aspects of this work. This research was supported by the National Institute for Deafness and Other Communication Disorders Grant PO1 DC-01837.

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