Sensory Cells Determine Afferent Terminal Morphology in Cross-Innervated Electroreceptor Organs: Implications for Hair Cells

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Type I and type II hair cells of the vestibular system are innervated by afferents that form calyceal and bouton terminals, respectively. These cannot be experimentally cross-innervated in the inner ear to determine how they influence each other. However, analogous organs are accessible for transplantation and cross-innervation in the brown ghost electric fish. These fish possess three types of electroreceptor organs. Of these, the sensory receptors of the type I tuberous organ are S-100- and parvalbumin-positive with a calbindin-positive afferent that forms a large calyx around the organ. Neither the sensory receptors nor the afferents of the ampullary organs label with these antibodies, and the afferent branches form a single large bouton beneath each receptor cell. In controls, when cut ampullary afferents reinnervate transplanted ampullary organs,

they have characteristic calbindin-negative terminals with large boutons. When type I tuberous afferents reinnervate ampullary organs, receptor cells remain S-100- and parvalbumin-negative, and the tuberous afferents still express calbindin. The nerve terminals, however, make large ampullary-like boutons on the receptor cells. These results suggest that (1) afferent terminal morphology is dictated by the receptor organ; (2) expression of calbindin by the afferent is not suppressed by innervation of the incorrect end organ; (3) ampullary organs generate ampullary receptor cells although innervated by tuberous afferents; and (4) ampullary receptor cells can be trophically supported by tuberous afferents.

Key words: tuberous organ; ampullary organ; hair cells; vestibular; cochlear; electric fish

Vestibular organs possess two types of hair cells with different morphologies and ion conductances (Wersäll, 1956; Correia and Lang 1990; Rennie and Ashmore, 1991; Eatock and Hutzler, 1992; Lapeyre et al., 1992; Baird et al., 1993; Rennie and Correia, 1994; Brichta and Goldberg, 1996). Type I hair cells are innervated by a large-diameter axon that envelops them within a calyx, whereas type II hair cells are innervated by a thinner axon that forms small bouton terminals (Wersäll, 1956; Favre and Sans, 1979; Peusner et al., 1988). How does the specificity between hair cell type and afferent terminal type come about during development? The alternatives are that afferents induce hair cells to differentiate into one type or other; hair cells direct the differentiation of afferent terminals; and the identities of both are predetermined and selectively accept each other. These hypotheses could be tested directly by switching the innervation of one cell type onto the other and observing whether afferents were accepted or rejected by the hair cell and, if accepted, whether the hair cell or the afferent terminal switched phenotype. The close proximity of the two types of hair cells within the vestibular epithelia makes this experiment impractical. It can be done, however, using the anatomically analogous electroreceptors of a weakly electric fish, the brown ghost (*Apteronotus leptorhynchus*) (Fig. 1).

Weakly electric fish generate electric fields to locate surrounding objects and for social communication (Bullock and Heiligenberg, 1986). These electric fields are detected with specialized receptor cells, called tuberous electroreceptors. There are two subtypes of tuberous receptors in this species. Type I receptors are innervated by a large-diameter afferent that forms a calyx around the receptor organ (Szabo, 1965; Zakon, 1987). Type II receptors are innervated by a small-caliber bouton-forming afferent. These fish also possess a second type of electroreceptor, ampullary electroreceptors, that are sensitive to low-frequency and DC electric fields (Szabo, 1965; Zakon, 1987). Ampullary afferents are smaller in diameter and form large bouton endings beneath each ampullary receptor cell.

Both tuberous and ampullary electroreceptor organs are found throughout the skin of the fish (Carr et al., 1982), and small patches of skin containing only a few receptor organs of known type may be removed and transplanted. In this study we transplanted a small patch of skin containing tuberous and ampullary organs into an ampullary organ-free region of skin, allowing the ampullary organs to be innervated by tuberous afferents. We found that the ampullary organs continue to generate ampullary receptors despite being innervated by a tuberous afferent, and that tuberous afferents form ampullary afferent-like endings, although the afferent continues to express tuberous afferent-specific markers.

MATERIALS AND METHODS

Surgical procedures. Brown ghosts were anesthetized with 3-aminobenzoic acid ethyl ester (Sigma, St. Louis, MO) (1:1000). The cheek and head were viewed under a dissecting microscope to locate regions of skin with the appropriate distribution of receptor organs. A small patch of ampullary organ-free skin on the cheek or the head (~0.5–2 mm²) was cut out with the tip of a scalpel blade and placed into a vial with 4%

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Figure 1. Schematic illustration of four logically possible experimental results from cross-innervation of an ampullary organ with a calyx-forming tuberous type I afferent. 1, The axon will be rejected. 2, The ampullary organ will induce the tuberous afferent to form bouton endings. 3, The tuberous afferent will induce the ampullary organ to become a tuberous organ. 4, The tuberous axon will form a large tuberous calyx on the ampullary organ.

paraformaldehyde for later histological analysis. A second patch of skin containing a cluster of ampullary organs was removed from the top of the head and transplanted into the hole left by the removal of the first patch. This group is designated the cross-innervated group. A second group of fish had two ampullary organ-bearing patches removed from the head and switched into each other's place. This was to control for any non-specific effects of the surgery on reinnervation. This group is designated the control group. The transplanted pieces of skin were held in place for a few minutes after surgery and usually remained in place. After surgery fish were revived with aquarium water and returned to their aquariums.

One week after surgery, fish were reanesthetized, and the cheek patch was viewed under the microscope to be certain that the transplant, the borders of which were demarcated by a wound margin in the epidermis, was still visible. At 2, 3, 4, or 6 weeks after the transplant, a larger piece of skin surrounding and including the transplant was removed and fixed as above.

Immunocytochemistry. Skin designated for immunocytochemical examination was fixed for 2 hr, washed in PBS, and stored in PBS at 4°C until it was examined immunohistochemically. Skin pieces were dehydrated in an alcohol series, defatted in methyl salicylate, and rehydrated. They were then brought through an acetone series to reduce nonspecific fluorescence and rehydrated. Skin patches were washed in PBS and incubated in PBS–1.0% bovine serum albumin–1.0% Triton X-100 solution and placed in primary antibody (Ab) overnight at 4°C. The next day they were washed in PBS and placed in secondary Ab for 1 hr (1:200 dilution; Cappel, Durham, NC), washed in PBS, and mounted on slides with gel mount. Pieces of skin that were double-labeled were simultaneously incubated with both primary Abs overnight and with both secondary Abs for 1 hr the next day. The sections were examined with epifluorescence optics.

The following primary antibodies and dilutions were used: 3A10 (1:1, a monoclonal antibody; Developmental Studies Hybridoma Bank, University of Iowa, Iowa City, IA) that recognizes a neurofilament-associated protein and serves as a marker for neurites; R8701 and R302 (1:200, polyclonal antibodies; kindly donated by Dr. Kenneth Baimbridge, University of British Columbia) against the calcium-binding proteins calbindin-28 kDa and parvalbumin, respectively; and Z628, which recognizes the calcium-binding protein S-100 (Dako, Carpinteria, CA).

Organs in the skin surrounding the transplants and axons entering the transplants could be viewed clearly in whole mount. However, because of connective tissue at the base of the transplant, axons and organs within it could not be seen. The skin was trimmed down to the borders of the transplant, washed in PBS, cryoprotected in 20% sucrose in PBS overnight, frozen at –70°C in isopentane, and embedded in OCT compound

(Tissue-Tek). The skin was then cryosectioned at 20 μ m and mounted on microscope slides.

Electron microscopy. Skin that was to be examined with an electron microscope was placed on a Petri dish, flattened, fixed in 4% paraformaldehyde and 2.5% glutaraldehyde for 10 min, and then immersed in a small vial containing the same fixative solution. The next day, the skin was transferred to a buffer solution, post-fixed in osmium (2.0% OsO₄ in NaPO₄ buffer) for 1 hr, dehydrated in serial alcohols and propylene oxide, and embedded in Epon plastic (Polysciences, Warrington, PA). Ultrathin (~90 nm) tissue sections were cut with a diamond knife, stained with uranyl acetate and lead citrate, and examined and photographed with the Hitachi HU 11-E transmission electron microscope (from the Cell Research Institute, University of Texas, Austin, TX).

RESULTS

Normal anatomy of electroreceptor organs

Electroreceptor organs appear as protuberances of the epidermal layer into the dermis. The two classes of tuberous organs and the ampullary organs are distinct (Szabo, 1974; Zakon, 1986, 1987). Briefly, both types of tuberous organs are connected to the exterior environment by a canal filled with loosely packed epidermal cells. Tuberous sensory receptors are free-standing within the lumen of the organ and rise above a thin epithelial support cell layer. The type I tuberous organ is the largest (100–200 μ m diameter) and is innervated by a large-diameter (10–15 μ m) axon that forms a massive calyx around the organ (Figs. 2–4).

The type II tuberous organ is smaller (60–100 μ m diameter) but structurally similar to the type I organ in other regards (Fig. 2). It is innervated by a thinner axon (4–6 μ m diameter), the branches of which terminate in a small bouton (2 μ m) beneath each receptor cell (Fig. 3). Each tuberous organ is innervated by a single afferent, and each afferent innervates only one organ.

Ampullary organs are the smallest in diameter (30–40 μ m) and are connected to the exterior environment by a canal that is devoid of cells. They possess about 8–10 sensory cells per organ that are embedded within the support cell epithelium. These sensory cells are innervated by a thin axon (2–3 μ m diameter) that makes a large bouton (6–8 μ m diameter) ending at the base of each receptor cell (Figs. 2–4). These organs are clustered, and an afferent innervates all the organs in a cluster (Zakon 1984, 1987).

We found that the sensory cells and afferents of these organs can be further distinguished by their immunoreactivity to a panel of Abs against different calcium-binding proteins (Table 1). All tuberous sensory receptors labeled with Abs against S-100 and parvalbumin. Both classes of tuberous afferents labeled lightly with the parvalbumin Ab, but only the large calyceal afferent of the type I organ was labeled by the calbindin Ab (Figs. 5, 6). None of these Abs labeled ampullary receptors or afferents (Fig. 6).

Control procedures

Ampullary organ clusters were visible under a dissecting microscope, allowing us to target for removal patches of skin that appeared ampullary organ-free. Nevertheless, it was critical to verify this. All presumed ampullary-free patches were labeled with an antibody to neurofilament-associated protein to visualize axon terminals and viewed in whole mount; each patch contained tuberous organs of both types but no ampullary organs ($n = 24$). The patches removed from the face (2–11) contained an average of 6.8 ± 2.9 tuberous organs. The average distribution of each organ type was 3.5 ± 1.8 type I organs and 3.37 ± 1.6 type II organs per patch, which reflects their approximately equal distribution in this area of skin.

Figure 2. Cross-sections of electrosensory organs. *A*, Type I tuberous receptor organ. Note the large-diameter, heavily myelinated axon at the base of the organ (arrows) and the row of free-standing sensory receptor cells with prominent nuclei (asterisk) that line the lumen of the organ. The bouton endings at the base of each receptor cell are not visible at this magnification. *B*, Type II tuberous receptor organs are smaller and innervated by a thinner axon. The sensory cells also have prominent nuclei. The parent axon is not in this section, but some of its branches are evident below the organ (arrows). The boutons are also not visible at this magnification. *C*, Ampullary organs are clustered. Their sensory receptor cells have prominent nuclei and are embedded within a support cell layer. Note their large-diameter osmophilic boutons (arrow). *e*, Epidermis; *d*, dermis. The parent axon is not in this section. Scale bar, 30 μ m.

It was also critical to determine whether the surgical removal of the patch might sever ampullary axons that run through the patch but that terminate on distant ampullary organs. We tested this by removing a small patch and then immediately removing a large area of skin surrounding the hole, labeling it with the Ab to neurofilament-associated protein, and examining it in whole mount ($n = 10$ on the face; $n = 10$ on the head). In a few cases,

it could be seen that removal of the skin patch severed one or two axons of passage; in all cases these were axons innervating tuberous organs adjacent to the hole. As anticipated, no ampullary organs were observed close to the hole, and the axons of distant ampullary organs were never transected when the skin patch was removed. Thus, it is unlikely that the removal of the skin patch in subsequent experiments would have resulted in transection of

Figure 3. Cross-sections of tuberous types I and II and ampullary organ afferents labeled with 3A10 anti-neurofilament-associated protein. Note the large calyceal terminal of the tuberous *Type I* afferent, the thin wispy terminals of the tuberous *Type II* afferent, and the large synaptic boutons of the *Ampullary* afferent. Scale bar, 30 μ m.

Figure 4. Whole mount of a piece of skin with a tuberous type I calyx (top) and an ampullary afferent (bottom). The fluorescently labeled axons are partially occluded by melanocytes. Tuberous type I afferents wrap around a single organ, whereas ampullary afferents innervate a number of smaller organs indicated here by a clustering of boutons. Scale bar, 60 μ m.

ampullary axons. Because each tuberous organ is innervated by a single axon, the number of organs removed (2–11) plus the few axons to neighboring tuberous organs that were severed during skin removal (2–4) is a measure of the total number of tuberous axons severed (≤ 15).

In a previous study of electroreceptors in another species, innervation of electroreceptor organs by more than one afferent was frequently observed (25%) in large patches of regenerating

skin (Fritzsche et al., 1990). In this study, polyneuronal innervation of single organs was never observed in control or cross-innervated skin patches.

Morphology of afferent terminals in control transplants on the head

As a control, we examined the morphology and immunocytochemical profile of ampullary organs in skin patches that were transplanted where an ampullary organ had been previously and where an ampullary axon was therefore without a target organ. We removed two patches of skin from the head, each containing an ampullary cluster as well as an average of 6.0 ± 3.7 tuberous organs (2–11; average type I, 2.3 ± 1.9 ; average type II, 4.0 ± 2.0) and switched them ($n = 8$). Skin patches were removed 3 or 4 weeks after transplantation, then sectioned, labeled, and examined. The receptor cells of the ampullary organs looked normal, and their afferent fibers made large bouton synapses on them. However, in none of the cases (zero of eight) were these afferents calbindin-positive, although the calyces of all the tuberous type I organs (16 of 16) in the same sections were calbindin-positive (Fig. 7).

Morphology of afferent terminals in cross-innervated transplants on the face

Pieces of skin surrounding an ampullary transplant were removed at 2, 3, or 4 weeks after transplantation, double-labeled with 3A10 to visualize the axons and R8701 to label calbindin, and examined in whole mount. In no cases were ampullary organs located adjacent to the transplanted patch; the closest ampullary organs were hundreds of micrometers from the transplanted skin patch, and their axons did not run through or make collateral sprouts into the patch. Thus, innervation of the ampullary organs in the transplant was not caused by collateral sprouting from afferents of organs outside the patch.

The skin was then trimmed down to the transplanted patch using the wound margins around it as a guide, and the patch was sectioned. At 2 weeks ($n = 6$) two ampullary organs had not yet been reinnervated; these were devoid of sensory receptors, because the receptors depend on the afferents for trophic maintenance (Szamier and Bennett, 1973; Weisleder et al., 1994, 1996). Two organs, although still devoid of receptor cells but presumably recently reinnervated, had extensive terminal sprouts running into the sensory epithelium and around the outside of the organ. This exuberance of afferent terminals is similar to what is observed in initial innervation during development or regeneration of new electroreceptor organs in larval fish or hair cells in the mammalian ear (Echteler, 1992; Sobkowicz and Slapnick, 1992; Duckert and Rubel, 1993; Vischer, 1995; Puel et al., 1997). In two other cases in which afferents had presumably arrived at the organs earlier, sensory cells were present, and small (2–4 μ m) boutons could be seen forming at their bases. Six of seven afferents innervating these ampullary organs labeled with the calbindin Ab.

We followed afferents through serial sections to determine whether they form extensive collateral fibers en route to the organs. Afferents never showed collaterals but seemed to grow directly toward an organ. Even afferents that had not yet reached an organ appeared to be growing toward it in a directed manner, and little or no collateralization was observed along the axon, even at its leading edge.

In all cases ($n = 18$), at 3 and 4 weeks after transplant the ampullary organs had normal-appearing ampullary receptors and were innervated by axons with large bouton endings. Most of

Table 1. Distribution of calcium-binding protein antibody labeling in electroreceptors and their afferents

	Tuberous				Ampullary	
	Type I		Type II		Rec. cell	Axon
	Rec. cell	Axon	Rec. cell	Axon		
Calbindin	0	+	0	0	0	0
S-100	+	0	+	0	0	0
Parvalbumin	+	+	+	+	0	0

Rec. cell, Receptor cell; +, immunoreactivity; 0, no reactivity.

Figure 5. Whole mount of skin containing one tuberous type I and two tuberous type II afferents double-labeled with 3A10 for neurofilaments (*top panel*) and R8701 for calbindin (*bottom panel*). Only the type I afferent expresses calbindin. Scale bar, 60 μ m.

these afferents (17 of 18) were calbindin-positive (Fig. 8). Thus, regenerating tuberous afferents only make large bouton endings on ampullary organs.

Tuberous organs in the transplanted patch were also examined to determine whether regenerating afferents made appropriate endings for each target organ (identified by the size of the organ), or whether they made large bouton ampullary-like endings indiscriminately. We found that afferents to type I organs made calyceal endings, and those to type II organs made thin bouton endings (data not shown).

Figure 6. Cross-section of an ampullary organ labeled with 3A10 for neurofilaments (*top panel*) and R8701 for calbindin (*bottom panel*). The ampullary afferents do not express calbindin. However, calbindin is expressed by large-diameter type I afferents running in a fascicle below the ampullary organ. Scale bar, 60 μ m.

In another set of cross-innervated patches examined at 3 and 4 weeks after transplantation, we labeled alternate sections with Abs against S-100 and parvalbumin to see whether the transplanted ampullary receptor cells now expressed these antigens. Although tuberous receptor cells were strongly labeled by both Abs (35 of 35 organs in eight fish) as in control fish, ampullary receptor cells were never labeled by these Abs (zero of eight) (Fig. 9).

The boutons of transplanted ampullary organs were also examined with the electron microscope. These boutons had normal-looking synapses in which vesicles in the receptor cell are clustered around an electron-dense synaptic ribbon that makes a finger-like projection into the bouton (Fig. 10).

Morphology of afferents in cross-innervated transplants on the head

Because the control transplants were made on the head, and most of the cross-innervated transplants were made on the cheek, we wished to be certain that there were no location-dependent dif-

Figure 7. Cross-sections of two different ampullary organs from two different fish reinnervated by ampullary afferents. These organs are double-labeled for neurofilaments (*NFIL*) and calbindin (*CALB*) and are presented in pairs (*A, B; C, D*). Note that the afferents do not express calbindin (*B, D*). Scale bar, 30 μ m.

ferences in reinnervation. We therefore made an additional set of cross-innervated transplants on the head. These were examined at 3 or 4 weeks after transplant and showed typical ampullary boutons, most of which (five of seven) were calbindin-positive (data not shown).

DISCUSSION

Calcium-binding proteins in tuberos receptor cells and afferents

Tuberos receptor cells labeled with Abs against S-100 and parvalbumin, whereas ampullary receptor cells did not. Vestibular and cochlear hair cells of most vertebrate groups contain various calcium-binding proteins (Rabié et al., 1983; Oberholtzer et al., 1988; Saidel et al., 1990; Demêmes et al., 1992, 1993; Dechesne et al., 1994) (W. M. Roberts, personal communication). Both classes of tuberos afferents also labeled with the parvalbumin Ab. In a striking parallel with the mammalian vestibular periphery in which only the large-diameter calyx-forming vestibular axons label with Abs against calretinin (Demêmes et al., 1992; Dechesne et al., 1994; Leonard and Kvetter, 1996), only the type I tuberos calyceal afferents labeled with the calbindin Ab. Ampullary afferents were negative for all of these markers.

The presence of calbindin in the type I tuberos afferent was not unexpected, because the type I tuberos receptors of *Apteronotus* fire phase-locked at 650–1000 Hz (Hopkins, 1976), and calcium-binding proteins are often observed in neurons with high-firing frequencies and sharp temporal accuracy (Takahashi et al., 1987; Braun, 1990; Baimbridge et al., 1992; Lohmann and Friauf, 1996). The type I afferents are the first stage in a “rapid-transmission, low-jitter” pathway that in-

cludes the spherical cells of the electric lateral line lobe and the giant cells of the midbrain torus semicircularis (Carr and Maler, 1986) that also label with the calbindin Ab (Maler et al., 1984). The central targets of the tuberos type II and ampullary afferents do not label with this Ab.

The idea that sensory neurons that express calbindin or calretinin participate in a rapid-transmission, low-jitter pathway in electric fish is further supported by a phylogenetic analysis: neurons in the time-coding pathway of *Eigenmannia* that phase lock at frequencies of 250–600 Hz label with a calbindin Ab, whereas those in *Sternopygus* that fire at modest frequencies of 50–150 Hz do not (Losier and Matsubara, 1990). Additionally, in mormyri-form electric fish calretinin is prominently expressed in many neurons in the time-coding pathway and expressed weakly or not at all in neurons not receiving strongly phase-locked input (Friedman and Kasawaskai, 1997).

Electroreceptor sensory cells dictate afferent terminal morphology; afferents do not influence sensory cell identity

Our results suggest that when tuberos type I axons reinnervate transplanted ampullary organs, the terminal morphology of the afferent is determined by the ampullary receptor cell rather than being intrinsically determined by the afferent. This conclusion depends on proving that (1) ampullary organs were not reinnervated by ampullary axons; (2) calbindin is a selective marker for tuberos type I afferents; (3) tuberos type I axons do not form ampullary-like endings on all sensory cells whenever they regenerate; and (4) the ampullary-like terminals

Figure 8. Cross-sections of three ampullary organs from three different fish reinnervated by tuberous afferents. These organs are double-labeled for neurofilaments (*NFIL*) and calbindin (*CALB*) and are presented in pairs (*A, B; C, D; E, F*). Note that the afferents strongly express calbindin yet show a typical ampullary large-bouton terminal morphology. Scale bar, 30 μ m.

are not a transient step en route to the formation of a tuberous type I calyx.

First, because the patches of skin that were removed for transplant never contained ampullary organs, ampullary axons were not severed during patch removal, and ampullary axons from neighboring organs did not supply collaterals to the transplanted patch, it cannot be the case that ampullary organs were reinnervated by ampullary axons.

Second, because none (zero of eight) of the axons innervating ampullary organs in the control transplants was calbindin-positive, we can conclude that ampullary axons do not express this epitope during regeneration. This is important because some calcium-binding proteins are transiently expressed during development or regulated by activity (Braun, 1990; Baimbridge et al., 1992; Dechesne et al., 1994; Philpot et al., 1997).

Third, we found that tuberous afferents that contacted

Figure 9. Cross-section of an ampullary organ (*A, B*) and a tuberos type I organ (*C, D*), each reinnervated by a tuberos type I afferent and labeled for neurofilaments (*A, C*) or S-100 (*B, D*). Note that the sensory receptors of the tuberos organ express S-100, whereas those of the ampullary organ do not, although reinnervated by a tuberos axon.

tuberos organs made tuberos-type, and never ampullary-type, terminals. Thus, regenerating tuberos afferents do not by default make large bouton synapses on all cell types and do not appear to make large bouton terminals even transiently on tuberos organs.

Last, even 6 weeks after transplantation (~3 weeks after initial innervation), tuberos axon terminals still show an ampullary-like morphology, demonstrating that this is not a transient occurrence. Interestingly, the tuberos axons still express calbindin at this time. At least judging by this one marker, then, there is not a wholesale switching of phenotype, suggesting that the transformation occurs locally within the cytoskeletal matrix of the afferent terminal. We conclude that afferent terminal morphology is dictated by the sensory receptors even when the afferent previously had a different phenotype and continues to express at least one aspect of that phenotype (calbindin immunoreactivity) (Fig. 11).

In addition, the fact that ampullary receptor cells cross-innervated by tuberos axons still look like ampullary receptor cells and do not express S-100 or parvalbumin suggests that the afferents do not influence the identity of the sensory receptor cells.

Tuberos axons trophically maintain ampullary receptors

Denervation of ampullary and tuberos organs results in the degeneration of the sensory receptors within either days or weeks (Roth and Szabo 1969; Szamier and Bennett 1973; Weisleder et al., 1994). New receptor cells may be generated from support cells at the base of denervated organs, but these receptors will also soon die if they lack trophic support (Weisleder et al., 1994, 1996). The presence of healthy ampullary receptor cells in ampullary organs reinnervated by tuberos afferents shows that tuberos afferents can provide trophic support for ampullary receptors (Fig. 11). It would be interesting to test whether afferents of the mechanosensory lateral line or other sensory fibers have the capacity to support the electroreceptive organs. There is some precedence for this in that denervated lateral line hair cells are saved from death when innervated by spinal nerves and when vallate taste buds, normally innervated by the glossopharyngeal nerve, are maintained by nerve fibers of the chorda tympani that normally do not innervate vallate papillae (Corwin et al., 1989; Oakley, 1993).

Figure 10. Electron micrographs of a normal ampullary receptor cell and one reinnervated by a tuberos afferent. *A*, A normal ampullary receptor cell with its large terminal bouton (asterisk). *B*, An ampullary receptor cell in an organ that had been reinnervated by a tuberos afferent and with an ampullary-like terminal (asterisk). *C*, A higher-power micrograph of a typical ribbon synapse. *D*, A developing ribbon synapse at a regenerating tuberos afferent→ampullary receptor synapse. Scale bars: *A*, *B*, 2 μ m; *C*, *D*, 400 nm.

Comparison with hair cells and their afferents

The calyceal terminals of the tuberos afferents and large bouton endings of the ampullary afferents are most similar to the types I and II vestibular afferent terminals, respectively. The associations between types I and II vestibular hair cells and their afferent endings were originally defined by electron microscopy (Wersäll, 1956; Favre and Sans, 1979; Peusner et al., 1988). In these studies, it appeared that types I and II afferents each innervated distinct hair cell types. However, electron micrographs do not lend themselves to reconstructions of whole terminal arbors, and recent reconstructions of horseradish peroxidase-filled vestibular afferents have revealed a large population of “dimorphic” afferents that simultaneously make calyceal endings on type I hair cells and bouton endings on type II hair cells (Fernández et al. 1988; Brichta and Goldberg 1996). This is in keeping with our results and emphasizes the power of the sensory receptor to dictate terminal morphology.

Our other main finding, that the identity of the sensory afferent is independent of its innervation, is in agreement with results showing that types I and II vestibular or cochlear inner and outer hair cells, which are distinguishable by nerve-independent criteria such as ionic conductances and hair bundle morphology,

differentiate normally in the absence of innervation (Corwin and Cotanche, 1989; Fritzsche et al., 1996; Lysakowski et al. 1996; Rusch and Eatock 1996). However, our study is the first to test whether the phenotype of the receptor could be shifted after cross-innervation of a hair cell-like receptor by an inappropriate afferent.

Implications for central maps

Tuberos and ampullary afferents project onto different regions of the brainstem. Tuberos afferents trifurcate and innervate three separate maps, whereas the ampullary receptors innervate a distinct ampullary map (Heiligenberg and Dye, 1982). Both regions map the body surface somatotopically. In addition, these afferents synapse on distinct cell types. Type I afferents project to spherical cells, and ampullary afferents project to pyramidal cells (Maler et al., 1981; Mathieson et al., 1987). It would be interesting to know whether the conversion of the afferent terminal influences the choice of the afferent for a postsynaptic target. Does a tuberos type I afferent, forced to innervate an ampullary receptor, remain on its normal target cell within a tuberos maps, or does it move about and come to innervate a pyramidal cell in the ampullary map?

Figure 11. Summary diagram of the relationship between receptor cells, support cells, and afferent neuron. It is hypothesized that the identity of the receptor cell is determined by the support cells that give rise to it because receptor cell identity is not influenced by cross-innervation. Because sensory receptors of denervated organs die but are healthy in cross-innervated organs, a tuberous afferent can support an ampullary receptor cell. Last, it is hypothesized that the receptor cell can dictate the morphology of the afferent terminal. The fact that tuberous afferents still express calbindin suggests that the effect on the afferent is a local one at the terminal.

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