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Cochlear transduction: an integrative model and review

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Abstract

A model for cochlear transduction is presented that is based on considerations of the cell biology of its receptor cells, particularly the mechanisms of transmitter release at recepto-neural synapses. Two new interrelated hypotheses on the functional organization of the organ of Corti result from these considerations, one dealing with the possibility of electrotonic interaction between inner and outer hair cells and the other with a possible contributing source to acoustic emissions of cochlear origin that results from vesicular membrane turnover.

Keywords

silent current; synaptic mechanisms; vesicular membrane recycling

A. Introduction

The orderly arrangement of receptor and supporting cells in the organ of Corti is a marvel of genetic coding and developmental processes. Cochlear hair cells possess several features that distinguish them from other labyrinth and non-mammalian auditory, vestibular and lateral line hair cells. While functional differences between the two types of cochlear receptor cells have yet to be established, abundant examples of morphological differences between them may be drawn from cytological, ultrastructural and histochemical studies. An integrative model of cochlear transduction is proposed that unifies the cell biology of hair cell synaptic transmission with the cytoarchitecture and electrophysiological properties of the organ of Corti. The model proposes that acoustic stimulation results in the shunting of an ionic current from outer to inner hair cells and includes a possible contributing source of non-linearities that are associated with the micromechanics of cochlear transduction. This source derives from changes in membrane surface area that accompany vesicular membrane recycling. The presentation of the model includes a review of those experiments relevant to its basic points and underlying rationale.

B. Inner hair cells

Precise rows of inner and outer hair cells (IHCs and OHCs, respectively) separated by the tunnel of Corti and contained within a spiralling bony capsule distinguish the organ of Corti from non-mammalian hearing organs. Mature cochlear hair cells are distinguished from vestibular hair cells by the absence of a kinocilium [7,28,60,131]. Cochlear hair cells are morphologically polarized by the presence of a cuticle-free portion of the cell's apical surface that in some species contains a basal body complex. Stereocilia of similar height are organized in a row with the tallest located furthest from the modiolus near the cuticle free zone.

Aside from a missing kinocilium, IHC morphology resembles that of mechanoreceptors found in vertebrate vestibular and lateral line organs. IHCs are flask-shaped with a bulbous lower portion, containing a large centrally located nucleus. IHC stereocilia form 2–4 curved rows which make tenuous contact, or no contact at all, with the tectorial membrane [52,53,90]. A complement of subcellular organelles characteristic of other eukaryotic cells is readily identified. Often a dense complex of rough endoplasmic reticulum and mitochondria may be found in the infranuclear region. The synaptic region is characterized by electron-dense presynaptic structures, variously known as synaptic bodies, bars, or ribbons, surrounded by clusters of vesicles [23,128,129]. Club-shaped stereocilia embedded in a cuticular matrix project from the apices of the hair cells. Stereocilia resemble large microvilli containing tightly packed microfilaments oriented longitudinally [73,131]. The microfilaments have been identified as actin filaments by their ability to decorate with myosin subfragment 1 (S1) [35, 151]. The actin microfilaments found in the acoustic papilla of the alligator lizard are so tightly cross-linked with adjoining filaments [511] that they cannot slide past their neighboring filaments without the expenditure of a considerable amount of energy to break and reform the chemical bonds that join them. Consequently, the stereocilia behave as rigid levers, as has been described for stereocilia of the frog crista ampullaris [36].

The membrane that envelops each stereocilium is continuous with the cytoplasmic membrane of the apical surface [3,29,151]. The apical membrane differs from the remainder of the cytoplasmic membrane in the size and distribution of intramembranous-particles [47]. The stereocilia and apical surface of the hair cell are exposed to endolymph and have a combined membrane surface area that varies between 15 and 20% of the total cell surface area (calculation based on the presence of 120 stereocilia [73], and height and radius measurements from Lim's review of cochlear anatomy [92]).

Hair cell transduction begins with deflection of the stereocilia bundle. Hudspeth and Jacobs [55] have demonstrated that the mechano-receptive ability of bullfrog saccular hair cells is unaffected by removal of their kinocilia. They suggest that the role of the kinocilium in vertebrate vestibular hair cells may be limited to the passive transmission of cupular or otolithic membrane movements to the stereocilia. The absence of a kinocilium in the receptor cells of the mature cochlea makes the sensitivity of the inner hair cells even more remarkable given the tenuous connection between their stereocilia and the tectorial membrane.

C. Outer hair cells

There are over three times as many of the slender, cylindrical OHCs as IHCs. The fact that the OHC nucleus is located basally in the soma is one of several differences between OHC and IHC morphology. The pronounced W pattern of OHC stereocilia contrasts with the bow arrangement of the rows seen on IHCs. Lim [92] has measured a progressive increase in mean length of stereocilia per hair cell from base to apex. A length increase also occurs in the radial direction with third-row OHC stereocilia longer than first-row OHC stereocilia. The radial trend is most dramatic in the apical turn, while stereocilia are of similar length for all three rows in the basal turn. Hair cell length follows a similar progression. A plot of hair cell length against mean stereocilia length reveals that the points fall about a straight line (values from Lim's graphical illustrations [92]), suggesting that the ratio of stereocilia membrane surface area to the remaining cell surface area is constant. With the exception of a missing kinocilium, IHC morphology generally resembles that of hair cells found in other systems; OHCs, in contrast, are distinguished by their relation to supporting cells, biochemistry, the presence of unique organelles called Hensen bodies, and an unusual synaptic complex. Each of these topics is developed more fully below.

Relation to supporting cells

Unlike IHCs, which are entirely surrounded by supporting cell (inner phalangeal cell) processes, OHCs contact supporting cells (outer Dieter cells) only at their apical and basal regions. Between these two points the greatest portion of OHC cytoplasmic membrane area is exposed to the fluid spaces of Nuel. Large extracellular compartments surrounding receptor cells are not found in other sensory organs, nor are they observed in the mature central nervous system where glial cells tightly envelop neurons. Glial cells mark the boundary of neuronal extracellular space, and are found within 30 nm of a neuron's cytoplasmic membrane [106]. The spaces of Nuel and the tunnel of Corti are filled with perilymph that is in free communication with Scala tympani [20,25] through channels in the basilar membrane [103]. The possible functional significance of large extracellular fluid volumes around OHCs has not been explored. One possibility is that diffusion of ions or metabolic substrates between the intra- and extracellular fluids of the organ of Corti is enhanced. Another possibility is that the absence of supporting cells permits unrestrained changes in OHC length. Kimura [74] has postulated that such freedom of movement would provide protection from possibly damaging movements of the basilar membrane. Still another possibility would be that a large extracellular space provides a buffer volume for the maintenance of a constant extracellular ionic milieu. One particular functional consequence would be to stabilize the extracellular calcium concentration, which could affect synaptic mechanisms at the recepto-neural junction.

Biochemical features

The OHCs possess a reticular network of subsurface cisterns in one or several layers, just inside that portion of their cytoplasmic membrane contacting the spaces of Nuel. Large mitochondria, elongated in the basal-apical axis, are found adjacent to the membrane lamellae and are relatively sparse near the cell's longitudinal axis in the supranuclear cytoplasmic region. Apically, lamellar membranes and mitochondria form a striking whorled complex, the Hensen body. The Hensen body is not present in IHCs, although similar structures are found in such diverse cell types as the Müller cell of the rabbit retina [97], lamprey meningeal cells [115], and neurons of the arcuate nucleus of the hypothalamus of castrated rats [6]. The Müller and meningeal cells contain large amounts of glycogen beta-particles and show glucose-6-phosphatase activity which, in the Müller cells, is specifically associated with the lamellar structures [96,115]. Both cell types are thought to be involved in glucose secretion.

Glycogen has been shown by both quantitative microchemical [148,149] and ultrahistochemical [24] methods to be present in relatively large quantities in OHCs and in very small quantities in IHCs. Duvall and Hukee [24] have shown that the glycogen is present as beta-particles. The beta form is typically found in metabolically active cells, such as kidney and cardiac muscle cells. The presence of glucosed-6-phosphatase has also been demonstrated in the organ of Corti [88]. Especially dense deposits of the histochemical reaction product were associated with the subsurface cisterns and Hensen bodies of the OHCs. It is not known whether the OHCs export glucose, or whether the metabolic demands of the OHCs themselves might require such an energy reserve and elaborate machinery.

Hensen bodies and secretory processes

The 'whorled bodies' of arcuate nucleus neurons, which appear and proliferate subsequent to castration [6], bear a close resemblance to OHC Hensen bodies. A sub-population of arcuate neurons secrete gonadotropin releasing hormones and are inhibited by gonadal steroids. Castration removes this feedback inhibition and increases the secretion of gonadotropin releasing hormones. Systemic injection of testosterone causes the incidence of whorled bodies to be greatly reduced, suggesting that their presence is correlated with increased secretory activity by the arcuate nucleus.

Hensen bodies exist in the normal OHC [3,133]. In instances of increased or abnormal demand, such as in aminoglycoside antibiotic intoxication [133], acute acoustic trauma [27], or prolonged exposure to moderate noise levels ([5]; see also electron micrographs in Thalmann [491]) proliferation of subsurface cisternal membranes and Hensen bodies has been reported. This response may not be merely a correlate of hair cell pathology, but an adaptive response to changing stimulus conditions. Lamellar membrane proliferation regresses with time, and only slight changes are apparent one month after stimulation [5].

Perhaps a related correlate of the effects of castration on cells in the arcuate nucleus is an increase in uptake of intravenously administered horseradish per-oxidase (HRP) into nerve terminals in the median eminence, the known projection area of arcuate neurons [136]. Increased HRP uptake has been associated with increased neurotransmitter release in a variety of systems [12,37,48,50,114,120,121]. Our own experiments indicate that OHCs display considerable HRP uptake in the absence of acoustic stimulation [8,9].

Synaptic morphology

The morphology of the synaptic region of OHCs differs between species and, in a given species, differs from IHC morphology [23,46]. Dense presynaptic bodies have been reported in the OHCs of guinea pigs [128,129] and monkeys [74]. However, investigators have noted that OHC synaptic bodies are smaller, or found less frequently, than IHC synaptic bodies in these species [1,74]. In the cat, the OHCs do not possess synaptic bodies [23,131] or aggregations of synaptic vesicles [23].

Electron-dense bodies surrounded by vesicles have been described for the presynaptic complex of mechano- and electroreceptors in eighth nerve and lateral line structures, for the retinal photoreceptor and bipolar cells (modified ciliated structures have been associated with both of these cells) and for the ‘ribbon fields’ found in the cytoplasm of pinealocytes (the pineal gland is a photosensitive organ in some non-mammalian vertebrates). Synaptic bars reach their greatest size (up to 3 μm long and 0.7 μm wide) and structural complexity in weakly electric fish [93,101,154]. Their role in the secretory processes of these cells has yet to be determined.

Studies on synaptic ribbons in retina [155] and ribbon fields in pinealocytes [75,150,153] indicate diurnal changes in ribbon density and number. In the cone photoreceptor of the fish retina, the number of synaptic ribbons decreases dramatically in the dark-adapted eye compared to the light-adapted eye [155]. Since neurotransmitter release in vertebrate photoreceptors is maximal in the dark [152] the disappearance of synaptic ribbons is associated with increased synaptic activity. Proteolytic digestion of ribbons after fixation suggests a significant protein component in their makeup [10,140], as does the absence of a lipid bilayer such as that surrounding other subcellular organelles. Schaeffer and Raviola [122,123] have recently demonstrated disaggregation and reaggregation of synaptic ribbons by manipulating the vesicular membrane recycling rates in turtle retina photoreceptors (for more details, see Section E).

Siegel and Brownell [125] have shown that synaptic bars can be easily found in chinchilla OHCs after the cochlea has been perfused with an artificial perilymph containing magnesium instead of calcium, followed by an aldehyde fixative having the same divalent cation substitution. Magnesium competes with calcium, resulting in reduced synaptic vesicle fusion and neurotransmitter release [94].

Further evidence for synaptic bar plasticity is provided by developmental studies in both the vestibular and auditory systems. Decreases in density and number of synaptic bars between fetal and mature Type1 vestibular hair cells have been reported [30]. Developmental changes of the recepto-neuronal synapse have also been observed in cat OHCs. Pujol et al. [112] report

that synaptic bars are abundant in the OHCs of fetal cats and become difficult to find shortly after birth. Tonic neurotransmitter release by OHCs would be increased by the depolarizing effect of a large endocochlear potential. The absence or presence of a very small fetal endocochlear potential may contribute to stabilizing fetal synaptic bars in OHCs. Kitten endocochlear potentials are less than + 10 mV on the first postpartum day and rise to adult levels within four weeks [31]. While fetal endocochlear potentials have not been measured, postnatal measurements in the marsupial show values of less than +5 mV until day 35, after which an increase similar to that seen in kitten occurs [124], suggesting that endocochlear potential is either non-existent or of very low magnitude in utero.

Differences in synaptic morphology may relate to functional differences between IHCs and OHCs. One interpretation of the morphological differences is presented by Gulley and Reese [46], who suggest that the OHCs may tonically release neurotransmitter, at least under the stimulus circumstances that prevail immediately prior to fixation in ultrastructural studies. The possibility of tonic neurotransmitter release by OHCs is also supported by the HRP experiments of Brownell et al. [8,9] and the magnesium substitution experiments of Siegel and Brownell [125].

D. Electrotonic interaction and the silent current

The presence of Hensen bodies, the absence of presynaptic bodies and vesicle accumulations in normally fixed material, and HRP uptake by outer hair cells in silence suggest that OHCs maintain neurosecretory processes at a high rate. High steady-state secretion of neurotransmitter at OHC recepto-neural junctions would require a depolarized resting membrane potential consistent with reported values [141]. Depolarization of OHCs could be maintained by a low-impedance stereocilia-apical membrane complex. The depolarizing current source originates in stria vascularis and is carried primarily by potassium ions [76,80, 117,118]. The importance of potassium ions in cochlear function is predicted from the existence of non-specific ion channel involvement in hair cell transduction and the high potassium ion content of endolymph [13].

In the vertebrate retina a depolarizing current of sodium ions flows between the synaptic and outer segment regions of photoreceptors. This current is called the ‘dark current’ because it is greatest in the absence of light [109,152]. Phototransduction leads to a decrement of the dark current with increasing light intensity. As a consequence photoreceptors are synaptically most active in darkness [114,120,121]. Our own observations suggest an analogous situation for OHCs. For this reason I have labeled the depolarizing potassium current of the cochlea the ‘silent current’ [8,9].

The silent current model of cochlear transduction is based on the modulation of the silent current across the organ of Corti. Electrotonic interactions between IHCs and OHCs have been proposed in previous cochlear models [98,139], or have been assumed by other models [21, 42,43] in order to account for the effects of stimulating the crossed olivocochlear bundle. The silent current model incorporates the electrotonic interaction proposed in earlier models but postulates that in the absence of acoustic stimuli, most of the ionic flow is through OHCs. Eighth nerve excitation requires IHC depolarization that could result when an impedance increase occurs at the stereocilia-apical membrane complex of OHCs and the silent current is shunted through IHCs, The lack of firm IHC stereocilia-tectorial membrane attachments suggests that at low stimulus intensities (the tip of eighth nerve tuning curves) virtually all IHC depolarization would be electrotonically mediated. As stimulus intensity is increased the greater excursions of the cochlear partition would cause direct mechanical stimulation of IHCs. Mechanical and electrotonic influences would interact until IHC mechanical stimulation dominates (tail of eighth nerve tuning curve and high-intensity CM generation by IHCs). The

postulated electrotonic interaction occurs via the subtectorial space, not by means of gap junctions (which are only found between supporting cells in the organ of Corti [45,61,62]).

The current-voltage relationships of saccular hair cells reveal that their membranes are approximately ten times more conductive when the intracellular potential is more positive than −50mV than when their potential is in the range −50 to −100 mV [13]. If IHC and OHC membranes show similar current-voltage relationships, an OHC whose membranes sit in the high conductance range at rest would show a smaller voltage change for a given current change than if it were sitting in the lower conductance range. A resting potential in the high conductance range would therefore cause the magnitude of intracellular receptor potentials to be smaller than would occur if the resting potential were in the low conductance range. The fact that OHC receptor potentials comparable to saccular [54] or IHC [116] receptor potentials have not been observed [141] would be consistent with the hypothesis that OHCs are normally depolarized. Maximum sensitivity to modulations of the depolarizing silent current would require that IHCs have resting potentials in the low conductance range of the current-voltage relationship. In this range a given change in current produces greater intracellular potential change than if the IHC membranes were in the high conductance slope. Higher resting potentials for IHCs [116] is consistent with this requirement. The parallel circuit arrangement of OHCs suggests that synchronous changes in their impedance would be required to shunt current radially towards the IHCs. The required synchrony would reduce the probability of spontaneous changes of a single OHC impedance from effecting a change in eighth nerve discharge. Increasing the impedance of an OHC stereocilia- apical membrane complex would cause its intracellular potential to become more negative, or hyperpolarized. An OHC hyperpolarizing response to acoustic stimulation would resemble the hyperpolarizing response of photoreceptors to photic stimulation. Possible hyperpolarization of OHCs in response to acoustic stimulation has been previously suggested [8,9].

Evidence for the possible sensitivity of IHCs to electrical current comes from experiments with electrical stimulation across the cochlear duct [83,147]. Current flow from Scala vestibuli to Scala tympani was found to increase activity in the eighth nerve while current flow in the opposite direction decreased the activity. Further evidence for the ability of hair cells to respond to both electrical and mechanical stimulation comes from work on the amphibian lateral line [138]. These experiments demonstrate that it is possible to mimic the effects of mechanical stimulation with electric currents. If the hair cells of the organ of Corti may be viewed as electroreceptors as well as mechanoreceptors, the possibility exists for electrotonic interactions between the IHCs and OHCs.

Excitatory direction of cochlear partition displacement

Konishi and Nielsen [77,78,79] achieved static deflections of the cochlear partition by blocking the helicotrema and displacing the oval window with trapezoidal stimuli. Their results indicate that excitation of auditory nerve fibers occurs when the cochlear partition is deflected toward Scala tympani and suppression during displacements toward Scala vestibuli. Excitation is accompanied by an increase in the endocochlear potential and suppression by a decrease. The same relation between auditory nerve activity and cochlear partition displacement has been observed in the intact gerbil cochlea [130,164].

The silent current model of cochlear transduction is consistent with the fact that cochlear partition displacement towards Scala tympani results in excitation of eighth nerve fibers. Basal displacement would cause stereocilia movement towards the modiolus, resulting in an increased impedance [54] and hyperpolarization of the OHCs. Silent current is shunted towards the IHCs, causing their depolarization and resulting in eighth nerve excitation. The extracellular consequence of an increase in OHC impedance would be a positive summating potential. Under the space clamped displacements of Konishi and Neilsen (77,78,79] the model would produce

a positive summating potential due to increased impedance at the apex of the more numerous OHCs. Another result of the silent current model is that the relation between outer hair cell synaptic activity and eighth nerve discharge is reciprocal. Reciprocity in function between the IHCs and OHCs is consistent with functional differences suggested by Zwislocki and coworkers [130,162,164,165] on the basis of single-unit studies of eighth nerve activity.

That auditory nerve fibers are excited by a Scala tympani displacement of the cochlear partition may seem contrary to what might be predicted when the Davis model [18,19] is coupled with the morphological polarization of cochlear hair cell stereocilia. Lateral placement of the tallest stereocilia in both the IHCs and OHCs would suggest, on the basis of functional polarization of hair cells [54,55], that movements deflecting stereocilia toward stria vascularis would depolarize hair cells while modiolar displacements would result in hyperpolarization. Displacement of the cochlear partition towards Scala tympani would deflect stereocilia towards the modiolus and result in a hyperpolarization of those cells whose stereocilia are firmly embedded in the tectorial membrane. IHC depolarization is required for eighth nerve excitation. That IHC, stereocilia deflection towards stria vascularis is inconsistent with Scala tympani displacement of the cochlear partition is another argument for the lack of a firm mechanical link between IHC stereocilia and the tectorial membrane.

Kiang et al. [70] have shown that for high click intensities eighth nerve fiber response latencies are shorter in response to rarefaction as opposed to condensation clicks. This information has been used by others [95] to infer that the excitatory direction for cochlear partition displacement is towards Scala vestibuli. While high-intensity response latency differences are clear, low-intensity response patterns are more ambiguous. An examination of Kiang's et al. data show that low intensity condensation click latencies often preceed those of rarefaction clicks. Peake and Kiang [108] show a similar intensity dependent change for the round window recorded N_1, response. N_1 shows shorter latencies for high-intensity rarefaction clicks, while low-intensity condensation clicks produce shorter latencies. Changes in the temporal order of eighth nerve responses are consistent with the possibility that electrotonic interactions dominate low-intensity eighth nerve response while high- intensity stimulation results in direct mechanical stimulation of IHCs.

Kiang et al. [70] also comment that in many low CF units with high rates of spontaneous activity the first response to low-intensity suprathreshold rarefaction clicks was a suppression of the spontaneous activity immediately before the first excitatory peak. The silent current model suggests that a small amplitude deflection of the cochlear partition towards Scala vestibuli results in deflection of OHC stereocilia away from the modiolus that causes a further decrease in the OHC impedance. More current would flow through the OHC pathway, resulting in a decrease of neurotransmitter release from the IHCs. The initial reduction in activity would give way to excitation as the cochlear partition moved towards Scala tympani. The incidence of an initial suppression of activity is not a common feature of the response to low intensity condensation clicks.

Crossed olivocochlear,bundle stimulation

The silent current' model is also compatible with experimental results of crossed olivocochlear bundle (COCB) stimulation [33,81,82,146,157]. These experiments consistently demonstrate that COCB stimulation causes a decrease in eighth nerve discharge, an increase in CM and a d.c. voltage shift. If the effect of efferent stimulation were to increase conductance at the base of the hair cell [33], the silent current model as well as earlier models of COCB action [42, 43] predict that more current would flow through the OHCs, further decreasing the current through the IHCs and causing them to be hyperpolarized. Desmedt and Robertson [21] have presented evidence that the effect of neurotransmitter release at OCB terminals is to enhance that portion of the current flow maintained by chloride ions. This would lead to a decrease in

synaptic activity at the IHC eighth nerve junction and decrease the discharge rate of eighth nerve fibers. The current increase through the OHCs would also be reflected in CM augmentation and a d.c. level shift.

Cochlear microphonics

Morphological observations that OHC stereocilia are more firmly coupled to the tectorial membrane than IHC stereocilia lead to the prediction, from the Davis model [18,19], that OHCs should produce a relatively larger receptor potential. Dallos and Wang [17] have recorded CM and SP from the cochleas of guinea pigs poisoned with kanamycin. Surface preparations showed an absence of OHCs in the basal turn. Both potentials were severely reduced when recorded in the basal turn. The CM input/output function was as much as 40 dB below normal. Pierson and Menler [111] have shown CM to have two components. The more sensitive, low-intensity response has a limited linear operating range and gives way under conditions of fatigue or hypoxia to a high-intensity component that is 180 degrees out of phase with the more sensitive response. Their conclusions are similar to those of Karlan et al. [63] whose arguments were based on round window recordings. They concur with Dallos and Wang's assignment of the more sensitive component to the OHCs, and suggest that the second component is generated by IHCs. By manipulating kanamycin dosage levels, Dallos and Cheatham [16] were able to obtain cochleas in which the basal turn showed normal IHCs, but no OHCs, and the apical turn shows no IHCs with some OHC retention. Records from the apical turn show minimal changes in CM and SP. Since the OHCs are responsible for virtually all of the potentials thought to be manifestations of receptor potentials, and since OHCs have very little direct control of eighth nerve activity [132], the authors suggested that OHCs may act to control current flow in the organ of Corti.

A portion of the Davis model postulates the existence of hair cell resting potentials on the order 'of −60mV. When added to the t; +80 mV endocochlear potential, a driving potential of 140 mV would exist across the apical surface of the hair cell. Russel and Sellick [116] report resting membrane potentials of between −20 and −43 mV for guinea pig IHCs. Tanaka et al. [141] show resting membrane potentials from histologically identified guinea pig OHCs ranging from −29 to −58 mV. In the same experiments they record negative potentials of greater magnitude from supporting cells. OHCs with the largest positive resting potentials were found in the second and third rows of OHCs. The presence of depolarized and positively polarized OHCs is consistent with a report that CM magnitude is not appreciably affected by Scala tympani perfusion with artificial perilymph containing a high potassium concentration [119]. These findings contrast with earlier reports [143,144] in which manual infusion of a high potassium concentration in a dilute mammalian Ringer solution results in reversible diminution of CM. The more recent experiments were careful to match perilymph ionic composition and used slow perfusion rates. If OHCs are the most significant contributor to CM, then the OHCs with conventional resting potentials would result in diminished CM as extracellular potassium depolarised the OHCs and decreased tile driving potential across the apical surface. The resting potentials reported by Tanaka et al. [141] would result in driving potentials across OHC apical membranes that are considerably less than the 140 mV postulated by Davis. Consequently, increasing perilymphatic potassium would have little effect on CM as most of the OHC cytoplasmic membrane 'rests' at a potential close to the potassium equilibrium potential predicted from endolymphatic and conventional intracellular potassium ion concentrations.

Calcium gradients and the spaces of Nuel

Present theories of neurotransmission require a high influx of calcium ions in order to result in a high rate of neurotransmitter release. Within the central nervous system information is carried over the length of an axon by means of an approximately 80 mV action potential. When the impulse invades the presynaptic axonal terminals, the resulting potential change causes an

influx of calcium and the subsequent release of chemical transmitter [64]. Within the central nervous system small changes in voltage near the resting level do not substantially affect release, providing a safety factor for synaptic transmission. On the other hand, hair cells, in a manner similar to other primary sensory receptors, do not generate sodium-potassium mediated action potentials. In these cells, changes of potential of less than a millivolt from the resting level can affect the discharge rate of postsynaptic cells. The presynaptic membranes of cells that signal in a graded fashion are likely to have properties different from the membranes of terminals invaded by all-or-none impulses: they must have either a novel mechanism for release, or they must be able to increase calcium influx in response to small voltage changes near rest. Morphologically unique presynaptic structures and evidence for the presence of voltage sensitive calcium channels in vertebrate hair cells [54] suggest both mechanisms may be active.

Measurements of extracellular calcium concentrations in cat cerebellum have shown that repetitive stimulation can cause a decrease from a base line of 1.2 mM to as low as 0.8 mM [107]. These changes were achieved by an unphysiologically synchronous stimulation of parallel fiber beams, suggesting calcium concentrations in the neuronal microenvironment may be quite stable under normal physiological conditions. Even if small changes in extracellular calcium were to occur in the CNS any effects on presynaptic calcium influx are reduced by the fact that presynaptic terminals are invaded by 80 mV action potentials. The sensitivity of hair cell synaptic release to small changes of the receptor potential suggests that maintenance of a constant calcium gradient is important for normal transduction with minimal adaptation. The same arguments for maintenance of a constant extracellular micro-environment are applicable for all ions normally present in low concentrations. Large fluid volumes would be ideally suited for the buffering of any substance found in low concentration and for which there is no chemical buffer. Potassium is maintained at a reasonably constant concentration within the CNS by what is effectively a large extracellular space created by glial cells whose membranes are highly permeable to potassium. Assuming the fluid that fills the spaces of Nuel and tunnel of Corti serves no other function than the maintenance of ionic concentrations, we must consider the possibility that the spaces exist for an ion other than potassium, as supporting or glial cells would be expected to be adequate for buffering of potassium. Because of the remarkable sensitivity of the auditory system, the maintenance of constant extracellular calcium concentration could be important, particularly if OHCs were normally depolarized so their voltage dependent Ca^{2+} channels were opened and local decreases in $[Ca^{2+}]$ might otherwise occur. Large extracellular volumes in the organ of Corti could help to stabilize extracellular calcium concentrations.

A possible role of the outer spiral fibers

Afferent fibers that innervate the OHCs exit from the habenula perforata follow a route along the base of the pillar cells across the tunnel of Corti to reach the area of the OHCs where they turn in a basal direction and form the outer spiral fibers between the Dieter cells. They spiral basally for 400 to 600 μ, gradually climbing towards the base of the OHCs where they make synaptic contact with between 10–20 of the receptor cells over the last 100–200 μm of their travel [110,131,132,134,135]. The radial fibers that innervate IHCs make contact with only one receptor cell. The difference in innervation pattern would suggest dramatically different physiological response characteristics for the two afferent fiber populations. Physiologists have been unable to find a unique class of eighth nerve fiber responses that constitute 5% of the total. An argument can be made that these fibers may not carry acoustically generated spike trains because of the considerable length (200–600 μm) the thin (0.5–0.8 μ diameter) unmyelinated outer spiral fibers travel prior to reaching the spiral osseous lamina. Information will travel towards the spiral ganglion either by means of action potentials or electrotonically. Action potentials have never been reported within the organ of Corti. While this is negative

evidence it is consistent with the possibility that the outer spiral Fiber resembles a dendrite and Na-K mediated action potentials do not occur along its length. If action potentials are assumed to be initiated at the first node of Ranvier in the spiral osseous lamina, then any postsynaptic signal will be severely attenuated as it is passively conducted toward the spiral ganglion. A postsynaptic event, passively conducted to a node 425 pm from the synapse will be less than 5% of its initial size*. Unless the outer spiral fiber membrane has a very large specific resistivity, it is likely that any possible postsynaptic signal would produce only subthreshold events for action potential initiation at the first node of Ranvier. Some invertebrate receptor cells have specific membrane resistivities that are two orders of magnitude greater than what is typically measured for mammalian CNS membranes [56] and show electrotonic conduction of receptor potentials with little decrement over 10 or more mm. The fact that specific resistivities measured for inner hair cells [116] are less than what is usually measured for CNS membranes argues against the possibility that membranes originating from cell bodies in the spiral ganglion are significantly greater. If outer spiral afferent fibers do not carry spike trains to the central nervous system, they may permit lateral interaction between OHCs. Nadol [104] has recently presented evidence from human material for the existence of reciprocal synapses on OHCs, opening the intriguing possibility for synaptically mediated lateral interactions between OHCs. Bodian [4] has described both filamentous and microtubular OHC afferent processes. The presence of both is consistent with Nadol's observations in that an afferent (microtubular) terminal on an OHC might give rise to an efferent (microfilamentous) terminal on a nearby OHC. The richness of possible lateral interactions is further enhanced by Bodian's [4] discovery of dendro-dendritic junctions between outer spiral fibers.

There is an example in another peripheral sensory receptor of synaptic interactions mediated by a portion of a neuron that is functionally separated from its soma. A population of horizontal cells found in the cat retina have two morphologically separate dendritic arborizations; the somatic aborization receives input predominantly from cones while a second arborization receives input predominantly from rods. The long thin process connecting the two units neither generates impulses nor allows significant passive electrotonic conduction between them [105].

Possible roles for the tectorial membrane

The protofibrils that make up the tectorial membrane [85] are unlikely to have a life span equal to that of the organism. In most biological structures regeneration occurs to replace aging components. The tectorial membrane and hair cell stereocilia are particularly subject to the mechanical stress associated with acoustic transduction. While growth of the tectorial membrane has been observed during development, the mechanisms that might maintain it have not been investigated. Cornford and Barajas [14] have recently demonstrated the incorporation of glucose into the cupula of *Xenopus* lateral line organs. The process occurs within two hours, and is preceeded by uptake in those supporting cells assumed to be responsible for generating the cupula. The possibility of a similar rapid turnover by the tectorial membrane could result in a tonic lateral deflection of OHC stereocilia and their tonic depolarization.

Evidence against this possibility comes from observations of normal appearing OHC stereocilia imprints in tectorial membrane for up to ten weeks after destruction of OHCs [57]. Either the tectorial membrane is a remarkably static structure or its regenerative mechanisms are under feedback control so that when a signal (perhaps contained in endolymph) has free access to the secretory cells in the spiral limbus, the secretory cells suppress their production of tectorial membrane. Another possibility is that Dieter cell phalangeal processes secrete the longitudinal

*A specific membrane resistivity of 1500 $\Omega \cdot cm^2$, and an axoplasmic resistivity of 100$\Omega \cdot$ cm results in a space constant of 137 μm in a 0.5 μm diameter fiber. The branching that occurs over the distance that synaptic contact is made would cause an additional signal decrement that has not been calculated.

TM protofibrils [85] which remain fixed to RL while radial protofibrils continue to grow from the spiral limbus.

Controversy exists over the nature of the fluid connections between the subtectorial space (i.e., between tectorial membrane and reticular lamina) and the endolymph. The marginal zone appears to anchor the tectorial membrane to the organ of Corti and is composed of a marginal band and marginal net. As its name might suggest, the marginal net has large holes through which even very large molecules could gain access to the subtectorial space. The marginal net has its greatest size in the apex and decreases basally until only the marginal band remains in turn one [84]. When the cochlea is fixed in a high-potassium, low-sodium medium, no holes are observed in the marginal band [86,87]. This observation led Kronester-Frei to speculate that the marginal zone might seal the subtectorial space from the endolymph. This conclusion is in contrast to the electrophysiological findings of Tanaka et al. [142]. They measured the potential in the subtectorial space and found it to be close to the endolymphatic potential. Manley and Kronester-Frei [99] have recently confirmed and extended an earlier report by Lawrence et al. [89]. They measured the potential of the inner spiral sulcus and found it to be the same as perilymph, which lead them to conclude that Hensen's stripe provides yet another boundary for the subtectorial space. An observation that supports endolymphatic origin of subtectorial space fluids is Flock's [34] electron probe analysis of cochlear fluids. He found the inner spiral sulcus to have a high potassium content, in the same ratio to chloride as found for endolymph.

Another critical question for cochlear function concerns the tectorial membrane's conductivity. The absence of a positive potential in the inner spiral sulcus argues against a conductive interface between the tectorial membrane and the fluids surrounding it. Partial electrical isolation of the subtectorial space would facilitate electrotonic interaction between hair cells particularly if the radial orientation of the constituent protofibrils imparts a conductive anisotropy. The presence of the tectorial membrane would greatly reduce the space constant for the resultant current pathway over the more open path that includes scala media. This would enhance the tuning capabilities of the receptor cell matrix as modeled by Stelioff [137].

In the absence of firm tectorial membrane attachment it has been hypothesized that subtectorial fluid displacement bends the IHC stereocilia by viscous drag. There is no direct evidence that relative movement between the reticular lamina and the tectorial membrane can bend IHC stereocilia [91]. Indirect evidence against the hypothesis comes from the static displacement experiments of Konishi and Neilsen [78,79]. If IHC stereocilia only respond to movement of the subtectorial fluid, then eighth nerve fibers should show a response only at the beginning and end of the trapezoid. Most eighth nerve units (67%) showed some tonic response. Those units that were only phasic tended to have low best frequencies; the investigators cautioned that low best frequency units innervate that portion of the cochlea nearest the damage caused in blocking the helicotrema and where the basilar membrane may not be undergoing static displacement from round window stimulation. The tonic response argues strongly against fluid displacement of IHC stereocilia. It suggests either direct mechanical stimulation by tectorial membrane or an electrotonic mechanism for IHC neurotransmitter release, or both.

E. Exo- and endocytosis; vesicular membrane turnover

The morphological evidence strongly supports the existence of a chemical synapse at the hair cell eighth nerve junction [23,46]. Any close opposition of hair cell–eighth nerve membranes characteristic of electrical synapses has so far escaped detection. Thin sections show the synapse to have all the morphological characteristics of a chemical synapse, including presynaptic densities, and aggregations of presynaptic vesicles. While the neurotransmitter has yet to be identified, there is now compelling physiological evidence in a hair cell organ that

the synapse is chemical. Intracellular recordings from afferent fibers innervating the hair cells of goldfish sacculus have demonstrated the quantal nature of excitatory postsynaptic potentials (EPSPs) [40,59]. The distribution of EPSP amplitudes and their quantal basis are consistent with other chemical synapses. Sound evoked EPSPs were phase-locked to the cochlear microphonic with a latency of 0.6–0.8 ms [39]. If the CM potentials are in fact receptor potentials for the hair cells then a latency of this magnitude is appropriate for a chemical synapse. Finally, the time course of EPSP amplitude shows a decrease after stimulus onset [41]. The last feature is particularly suggestive of a chemical synapse as similar adaptation has not been observed in hair cell receptor potentials recorded intracellularly [15,32,102,116]. Eighth nerve response to moderate or high intensity stimuli typically shows rapid adaptation to a steady discharge rate following an initial high discharge rate at stimulus onset. Since adaptation is present postsynaptically and not in the receptor potential the possibility of an electrical synapse is even more unlikely.

Neurotransmitter release at the neuromuscular junction may be vesicular, non-vesicular or a combination of both [11,145]. The possibility that synaptic transmission at the hair cell eighth nerve synapse is chemical but not vesicular must be entertained. The strongest evidence for vesicular release by hair cells in the organ of Corti is the presence of dimples and plasmalemmal deformations on the cytoplasmic leaflet of freeze-fracture material at the afferent synapses of both IHCs and OHCs [46]. Similar dimples and deformations are associated with vesicular fusion for other synapses. The presence of vesicular fusion structures argues that a portion of hair cell neurotransmitter release is vesicular. Further evidence comes from the presence of HRP in vesicles and multivesicular bodies in IHCs [20], and both IHCs and OHCs [Brownell, unpublished observations].

A growing body of evidence supports the concept that neurotransmitter release begins with a voltage-dependent change in membrane permeability to calcium resulting in an influx of calcium ions. This is followed by calcium-dependent binding of synaptic vesicles to release sites in the presynaptic membrane. Vesicles fuse with and collapse into the presynaptic membrane, releasing neurotransmitter into the synaptic cleft. The total surface area of the presynaptic membrane is increased in the process because of the addition of vesicular membrane.

Cell surface area is maintained within narrow limits by endocytotic recovery of vesicular membrane. Endocytosis begins with an invagination in the cytoplasmic membrane at a re-uptake site near the presynaptic release site. The invagination develops into a pit that seals off and the vesicle so formed is drawn into the cytoplasm. Recovered membrane is presumably utilized in the production of more synaptic vesicles and is an example of cellular recycling of essential molecules at great savings to the cell in terms of the energy that would be required to synthesize the material de novo. Controversy exists about specific aspects of stimulation–secretion coupling and vesicular membrane recycling. The evidence for many of the steps involved in membrane recycling as well as the remaining points of contention are presented in several recent reviews [11,22,26,49,51,65,145,161].

Changes in membrane flux at any stage of vesicle membrane recycling will necessarily result in modulation of cell surface area as a dynamic equilibrium between new rates of exo- and endocytosis is reached. Modulation of synaptic activity has been demonstrated to produce changes in cell surface area for a variety of neurons. Synaptically mediated changes in surface area have been demonstrated for turtle cone pedicles both in response to photic stimulation and reduction of temperature [123]. The latter manipulation disrupts normal membrane recycling by reducing endocytosis more than exocytosis, with the result that synaptic terminals exhibit greatly reduced vesicle density and increased cytoplasmic membrane surface areas. Light-induced modification of horizontal cell digitations into cone pedicles has been demonstrated

in fish retina [113,156]. The changes in synaptic morphology may be due to changes in vesicular membrane flux. Blackwidow spider venom, which increases release of neurotransmitter at the neuromuscular junction, also increases the volume of the motor endplate [38], presumably because endocytotic mechanisms cannot keep pace with the addition of vesicular membrane. Addition of vesicle membrane in axon terminals as a result of electrical stimulation has been demonstrated in the lamprey spinal cord [69]. The same mechanism may account for light microscopic observations of synaptic bouton enlargements on cat spinal cord neurons after tetanic stimulation of the dorsal root [58].

Acoustic energy from the cochlea: mechanical sequellae of synaptic activity

A microphone placed in the external auditory meatus can measure sound pressure originating in the cochlea. It occurs with a latency of between 5 and 30 ms following the presentation of a transient sound (click or 3 ms tone burst) [66]. Phase shifts [67,68] and distortion products [71,100,126,158] can also be detected and shown to originate in the cochlea [159]. These phenomena demonstrate that the cochlea is capable of generating acoustic energy as well as converting it to neural energy. Evidence for a receptor cell origin of cochlear acoustic emissions comes from the observation that mechanical distortion products measured in the ear canal can be modulated by COCB stimulation [100,126].

Wilson [160] has proposed that hair cell swelling could be the source of acoustic cochlear emissions. The mechanisms associated with vesicle membrane fusion and reuptake result in surface area changes that could permit hair cell volume changes. The possibility of surface area fluctuations similar to those reported at other synapses must be entertained for the hair cell, particularly in view of the large number of afferent synapses confined to the infranuclear regions. Ionic flow from endolymph to perilymph could produce rapid hair cell volume changes as the cell surface area is varied if bulk flow of water accompanies ion movement and the permeability of the cuticular plate-basal body complex is greater than the lateral and infranuclear cytoplasmic membrane. The synaptic or infranuclear region of an outer hair cell can be modeled as a hemisphere with a radius of 3 μm and the supranuclear region by a cylinder with a height of 12 μm. A net change of 100 synaptic vesicles, each with a diameter of 40 nm, results in a volume change of 0.5%. While values of vesicular flux are not known, it is possible to estimate an upper limit for the vesicular release rate that a single hair cell is capable of by examining postsynaptic eighth nerve firing rate. Approximately 20 radial fibers make synaptic contact with one IHC. Each fiber is capable of firing at sustained rates in excess of 150 spikes/s. If each vesicle fusion causes a single action potential, then an IHC can maintain a vesicular membrane flux of 3000 vesicles/s. A change of 100 vesicles/s seems well within possible limits.

Continual vesicle release by OHCs in silence could result in maximal cytoplasmic surface area of the OHC. A transient acoustic stimulation would result in a momentary reduction of vesicle release. If the vesicle membrane recycling mechanisms continue to operate at their usual high rate then the surface area of the OHC will be reduced and the cell will shrink in size. An observation that tends to support this mechanism is that intense acoustic stimulation results in a proliferation of smooth endoplasmic reticulum in the OHCs and the cells becoming flask shaped in contrast to their usual cylindrical appearance [5,27]. Shortly after the end of acoustic stimulation a cytoplasmic surface area rebound occurs as the ionic currents reestablish themselves. Increase in potassium current in the OHC will lead to increased vesicle fusion at the synaptic membrane, resulting in an increase in cytoplasmic membrane surface area, which in turn will allow the cell to return to its normal resting size. The postulated modulation of hair cell size results from the modulation of ionic flow through the hair cell. The source of the ionic flow is the stria vascularis, which provides continual endolymph production [80] and is therefore the candidate energy source for cochlear emissions as well as the silent current. Expansion of hair cells would set up vibrations in the cochlear partition that would be

transmitted back along the ossicular chain and set the eardrum in motion. In the case of transient acoustic stimuli the rate of expansion would be greatest at the beginning of the vesicle fusion process, the frequency of the emissions would be highest at that time and gradually taper off, consistent with experimental observations [66]. The latencies observed for cochlear emissions are longer than those associated with molecular processes such as muscle contraction. Pinocytosis has been postulated to be triggered by the aggregation of molecules common to vesicular membrane at the reuptake site [49]. The latencies associated with cochlear emissions may indicate the diffusion time of vesicular membrane molecules through the cytoplasmic membrane from the synapse to the reuptake site. The existence of continuous cochlear emissions in the frequency range below 5 kHz is compatible with synaptic mechanisms in the organ of Corti that permit phase locking in the eighth nerve response in the same frequency range.

While many descriptions of mechanical oscillations in the cochlea confine themselves to considerations of the basilar membrane (BM), more recent efforts [2,72,163] recognize that transduction begins by movement of hair cell stereocilia embedded in the hair cell cuticular plates, which in turn are part of the reticular lamina (RL). The mechanical properties of the basilar membrane and the process of acoustic transduction will be influenced by the micromechanics of the organ of Corti. The nature of the mechanical link between the BM and RL is a critical feature of mammalian auditory transduction. The apical ends of hair cells and supporting cells make up the RL. Tight junctions between apposed membranes in the RL provide structural integrity and a barrier to passive diffusion [45,61,62,127]. A secure mechanical coupling between the BM and the modiolar portion of the RL is afforded by the inner and outer pillar cells whose microtubular packed processes span the distance between their cell bodies on the basilar membrane and their interdigitating elements in the RL. Mechanical security is assured by the triangle (tunnel of Corti) formed by the BM and the struts of the inner and outer pillar cells. The basilar membrane attaches to the spiral osseus lamina (SOL) medially and the spiral ligament (SL) laterally and can be divided radially into two regions: pars tecta is modiolar, narrower, and thinner; pars pectinata is lateral, wider, and thicker [90,92]. The inner vertex of the triangular tunnel of Corti is near the point where the BM attaches to the SOL. The structural differences between the SOL and the BM suggest a mechanical discontinuity that would cause the triangular tunnel of Corti to pivot about its inner vertex as the cochlear partition vibrates. If the SOL and SL are relatively rigid, the point of maximum excursion for the BM in response to acoustic stimuli most likely occurs near the junction of pars tecta and pars pectinata. The basal portion of third row OHCs contact Dieter's cells at this location. In contrast, the base of the inner hair cells must undergo considerably less movement. They are cradled by supporting cells which in turn are anchored on the outer lip of the SOL. Movement of the lateral aspects of the RL is achieved either by the intrinsic rigidity of the RL, as suggested by Smith [127], or by direct mechanical coupling between BM and lateral RL. The geometric arrangement between OHCs and their supporting cells may determine the degree to which BM movement leads to OHC stereocilia movement.

Outer Dieter cells feature a connecting element between their nuclear region on the basilar membrane and their phalangeal process in the reticular lamina. The outer Dieter cell's phalangeal process interdigitates between the cuticular plate of the two basally adjacent OHCs. The connecting element differs from the struts of the pillar cells in that the density of fibrils that might impart rigidity is considerably less. Even if the connecting element were rigid the fact that it slants basally would make the establishment of a firm mechanical link between the BM and RL difficult for it alone to provide. The OHCs tilt apically at about the same angle as the Dieter cell connecting elements angle basally. An OHC and the connecting element of the Dieter cell on which it sits form the sides of an inverted isosceles triangle whose vertex is the point of contact between the hair cell and its Dieter cell. The base of this triangle is that portion of the RL between the cuticular plate of the hair cell and the Dieter cell's phalangeal process.

Only if the OHCs themselves are stiff can a structurally rigid unit be formed. Any variance in OHC length or rigidity would affect the mechanical coupling between BM and lateral RL. If either of these factors were to vary dynamically in response to acoustic stimuli, then the micromechanics of the organ of Corti and consequently the transduction process would be altered. The micromechanical results of modulation of vesicular membrane turnover may provide the negative mechanical damping postulated by Kim et al. [72] in their recent models of cochlear mechanics.

Electrical changes resulting from synaptic activity

A change in membrane area also modulates the total membrane resistance, independent of cell volume changes. If a specific resistivity of 1000 Ω cm^2 is assumed then the basal hemisphere of the model hair cell experiences a resistance change of 15 MΩ for the same change in vesicle flux (100 vesicles). Small changes in total membrane capacitance also occur (< 0.1 pF, assuming a specific capacitance of 2 μF/cm^2) that would result in small changes of the membrane time constant of the receptor cell. Impedance changes that might occur from changes in the cell's surface area would produce intracellular potential changes in the same direction as those initiating the change in vesicle membrane flux. A depolarizing potential that increases vesicle fusion and results in an increase of cell surface area further depolarizes the cell by increasing membrane conductance. Conversely, a hyper- polarizing response decreases cell surface area, leading to an impedance increase that further hyperpolarizes the cell. Also working synergistically with changes in the cell surface area at the base of the hair cell are the micromechanical effects at the apical end that result from possible changes in hair cell length. A consequence of any shrinkage would be to further straighten the stereocilia of the OHC which would further increase the impedance.

F. Conclusions

The silent current model of cochlear function is based on a number of unproven but testable assumptions. One of its pivotal hypotheses is the existence of a hyperpolarizing d.c. component in the OHC response to acoustic stimulation. The establishment of this component, in concert with Russel and Sellick's [116] demonstration of a depolarizing d.c. response for IHCs would prove that IHC and OHC responses are reciprocal in nature. Reciprocal d.c. responses would not in themselves demonstrate the postulated electrotonic interaction. The most direct test would be simultaneous recordings from inner and outer hair cells during current injection into Scala media or into an inner or outer hair cell. Given the reported difficulty in achieving stable recordings in a single receptor cell in the organ of Corti, simultaneous recording from two would require considerable technical skill, particularly if the presence of the electrodes were not to effect the mechanics of the cochlear partition.

A possibly less difficult procedure may be to measure the silent current. Proof that the dark current exists in the retina was based on current density measurements [109]. Current density is the product of the first spatial derivative of electrical potential and the conductivity tensor. The discontinuous conductivity of the organ of Corti precludes a simple interpretation of current density immediately surrounding the hair cells. However, knowledge of current density in the fluid spaces immediately above and below it should permit a reconstruction of the distribution of ionic flow through the cochlear partition. One pitfall of this approach may be that the high conductivity of the cochlear fluids would result in extremely small potential gradients. If successful, current density measurements may permit a demonstration of the postulated shunting of the silent current between inner and outer hair cells.

The origin of cochlear emissions in vesicular membrane turnover may not be amenable to direct proof. Supporting evidence may be gained if cochlear emissions are proven labile to the same manipulations that are known to affect synaptic membrane turnover. Direct proof would require

morphometric analysis of hair cells in stimulated and unstimulated conditions. Chemical fixation techniques are much slower than cochlear synaptic events. Even if the freezing agent could be administered directly into the bony labyrinth, existing freezing methods are much slower than cochlear events. Rapid freezing techniques may one day be capable of stopping vesicular membrane turnover. An attractive alternative would be to utilize the isolated OHC preparation developed by Goldstein and Mizukoshi [44] to study the electrical properties of the OHC membrane in the same manner that isolated photoreceptors are currently being studied. Individual photoreceptors are gently aspirated into micropipettes permitting analysis of membrane characteristics over discrete portions of their surface. The predictions of electrical impedance change accompanying changes in vesicular membrane turnover may then be directly tested

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