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Overt and covert paths for sound in the auditory system of mammals

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Abstract

of the article “Overt and covert paths for sound in the auditory system of mammals”, by Bernard M. Auriol, Jérôme Béard, Jean-Marc Broto, Didier F. Descouens , Lise J.S. Durand, Frederick Garcia, Christian F. Gillieaux, Elizabeth G. Joiner, Bernard Libes, Robert Ruiz, and Claire Thalamas. (May 08, 2014)

The consensus, according to which the transmission of sound from the tympanum to the Outer Hair Cells is solely mechanical, is problematic, especially with respect to high pitched sounds. We demonstrate that the collagenous fibers of the tympanum produce electric potentials synchronous to acoustic vibrations and that, contrary to expectations, their amplitude increases as the frequency of the vibration increases. These electrical potentials cannot be reduced to the cochlear microphonic. Moreover, the alteration of collagen as well as gap junctions between Deiters cells results in hypoacusis or deafness. The discovery of an electronic pathway, complementary to air and bone conduction has the potential for elucidating certain important as yet unexplained aspects of hearing with respect to cochlear amplification, otoacoustic emissions, and hypoacusis related to the deterioration of collagen or of gap-junctions. Thus, our findings have important implications for both theory and practice.

Summary

The collagenous fibers of the eardrum produce electric potentials synchronous to acoustic vibrations and their amplitude increases as the frequency of the vibration increases. This finding lends support to our hypothesis of an electric pathway of sound transmission.

Content

The piezo-tympanic signal

The tympanum and its environment

Sound waves present in the environment pass through the external auditory conduit and arrive at the tensed portion (pars tensa,) of the tympanic membrane (fig. S10 in SOM => will be further updated), which is made up of four layers. These are the epidermal layer (outermost layer), the external layer of the lamina propria, the internal layer of the lamina propria and the
mucosal layer (innermost layer).
The epidermal and mucosal layer act as shields for the lamina propria. The external layer of the lamina propria consists of circular collagenous fibers (fig. S11 in SOM => will be further updated), which originate at the manubrium, describe an arc around the umbo and rejoin the manubrium on the opposite side. The internal layer, made up of radial fibers of collagen ([1, 2, 3, 4, 5, 6, 7, 8]) is thicker (18 µm at its periphery, 7 µm at the center) than the circular layer ([6, 8, 9, 10]). The fibers of the radial layer go from the periphery of the tympanum to a central structure : the handle of the malleus and its extremity (umbo). These fibers play an important role in the transmission of the high-frequency sounds "unique to mammals" ([11, 12]). The tympanum is attached at its bony circumference by means of the annulus fibrosus, which helps to regulate its tension by means of an annular ring of radially oriented smooth muscles ([13]).
The tympanic membrane (TM) is a non-uniform structure[1] with varying mechanical properties as a function of position ([14]) and, as is the case for all stretched membranes, the eardrum has natural resonance frequencies that produce a fragmentation of its surface into vibrating zones[2]. This is interesting especially above 2 kHz ([15, 16, 17, 18, 19]). This being the case, the highest frequencies are not transmitted to the ossicles with great precision, and this apparent flaw in current theory has not been convincingly explained ([6, 20, 21]). "*The significant sound pressures measured at certain frequencies (eg 6 kHz) after ossicular interruption suggest that sound is transmitted to both [cochlear] scalae through a path independent of the ossicular chain*" ([22]).
Furthermore, the rubber-band-like action of the incus-stapes joint ([23]) and the trampoline-like action of the annular ligament of the stapedio-vestibular joint ([24]) do not produce the best possible transmission of high frequencies ([25]).The response time to acoustical stimuli, for some species, is below 5 µs. This is a surprising result, considering that biological processes are generally slow, with the shortest response time on the order of 1 ms (Harnagea, pers. com., 2012). Further, bone conduction does not mobilize the umbo with a sufficient velocity for the transmission of frequencies above 3 kHz ([26]).
The consensus, according to which the transmission of sound from the tympanum to the Outer Hair Cells is solely mechanical, is problematic especially with respect to high pitched sounds ([27, 28, 29, 30, 31, 32, 33, 34, 35, 36]). It seems, then, that a mechanism independent of the chain of ossicles is necessary for optimal transmission of high frequency sounds.

The collagen of the tympanum, a piezo-electric bio-electret

The fact that four types of collagen (I, II, III, IV) are present in the tympanum ([37]) has been well documented. The quantity of Type II collagen, especially in the radial fibers, is particularly noteworthy ([38]). This type of collagen, an essential constituent of cartilage, is found throughout the auditory system ([39]). Its role, still not

[1] "*Because of the difference in collagen fiber density throughout the TM, it is very likely that TM displacements relate to the structure and orientation of the collagen fibers*" (Thyden and Rutledge, 2012).
[2] The vibrations of an idealized circular drum head, essentially an elastic membrane of uniform thickness attached to a rigid circular frame, are solutions of the wave equation with zero boundary conditions.
http://www.sciences.ch/htmlfr/mecanique/mecanondulatoire01.php#modevibrationmembranetendue

completely understood, is considered critical for audition ([40]). Indeed, the genetic deterioration of this collagen produces deafness ([41, 42, 43, 44, 45]), especially for sounds above 3 kHz ([46]). Similarly, deafness can be produced by the aging of collagen II ([47, 48, 49, 50]) or its deterioration due to toxins ([51, 52 , 53]) or autoimmune disease ([54, 55, 56, 57, 58, 59, 60]).

The triple-helical collagen molecules are organized hierarchically into fibrils, fibers, and bundles (61). Fibers, like fibrils, are bioelectrets ([62]), having a negative pole (C) and a positive pole (N). The latter is the growth pole of the fiber ([63]) and the growth of the radial fibers of the tympanum occurs from the periphery toward the central area ([64]).The collagenous fibers of the radial layer of the tympanum are centered not only on the umbo but also all along the handle of the malleus ([38]) (See Line F of ???).

Collagen I fibers are piezoelectric ([65, 66]). Stimulating these fibers by means of high frequency sounds directly affects osteogenic cells ([67]) (morphogenic effect). And the production of the collagenous fibers of the eardrum is increased and modeled by acoustic stimulations: In vitro, applied mechanical forces are able to promote TM-fibroblastic differentiation, increasing the production of collagen type II that is a peculiarity of TM structure ([68]).

The piezoelectric tensor of collagen I has a symmetry close to the hexagonal crystal structure ([69]). A detailed analysis of the Piezoresponse Force Microscopy signal [of collagen I] "…*revealed clear shear piezoelectric activity*[3] *associated with piezoelectric deformation along the fibril axis*." [(70)]. Piezoelectric activity of collagen fibrils can be detected in vitro in a large range of frequencies going from a few Hz ([71]) up to more than 200 kHz ([70]). This result corresponds to the outcomes of several studies with respect to collagen in vivo ([72, 73, 74, 70]). The inverse piezoelectric effect is also demonstrable ([66, 70]). The properties of collagen type I are thought to be similar to properties of collagen type II ([66, 75,76, 77]) and we have measured synchronous electrical potentials on the patellar ligaments of individuals at various ages. The amplitude of measured synchronous potentials increases with the frequency of the sound signal and there is a strong correlation between measurement on one knee and on the other knee of the same subject (see SOM)

In vitro research with respect to collagen I piezoelectricity has consistently found the fibrils to be randomly oriented; i.e. one direction mixed with the other ([78])

This is somewhat in contradiction with the macroscopic in vivo measurements: if the fibrils are oppositely oriented (50/50), then the piezoelectric effect should be cancelled, but this is not the case. This suggests that globally, the number of fibrils oriented in one direction is greater than the number of fibrils oriented in opposite direction (Harnagea, pers. com., 2012).

Measurements in vivo of the piezo-electricity of the human tympanum

It is possible to detect an electric potential synchronous to the acoustic vibration between an indeterminate point of the tympanum and the mastoid bone ([79]). It does not follow necessarily, however, that the potential measured in this type of experiment is produced by the Outer Hair Cells (OHCs).

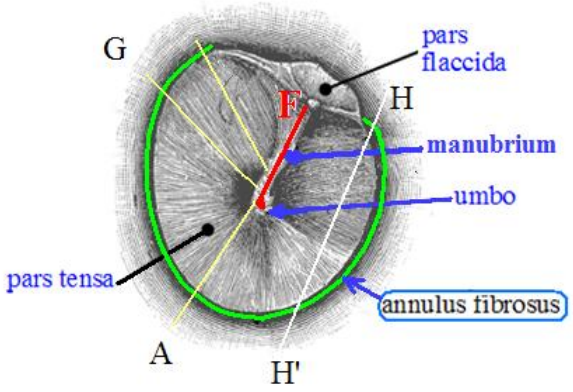
Our methodology ([80]) allows us to demonstrate, in vivo, and under normal physiological conditions, the piezoelectricity both of collagen I in tendons (see SOM, Measures on tendons : **Erreur ! Source du renvoi introuvable.**) and of tympanic collagen II.

[3] More than ten millivolts.

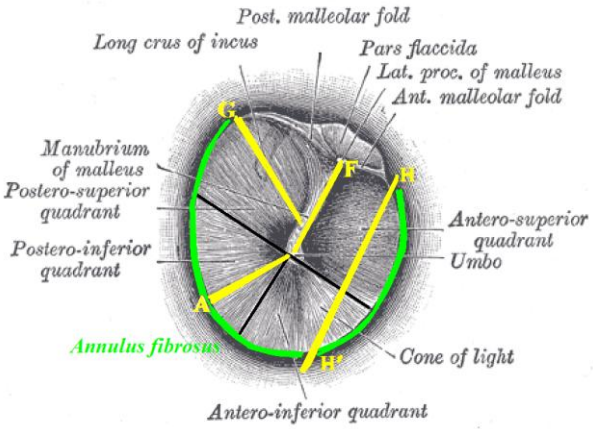
We use a lock-in amplifier to drive a loud speaker. In this manner, we broadcast a sinusoidal sound at about one meter from the external auditory conduit. We position a probe consisting of two electrodes at the center and the periphery of the tympanum. This probe captures the piezoelectric response of the radial tympanic fibers when they vibrate in response to the sound sent to the tympanum. The lock-in amplifier makes it possible to select only those electrical responses synchronous to the acoustic stimulation. We measure electrical responses to stimulations at different acoustic frequency levels[4]. The piezoelectric potentials in vitro can be above ten mV if the fibers share a homogenous direction and polarity; they are drastically reduced for a set of bundles which have opposite polarities. Likewise, the in vivo measurements are substantially lower, probably partly because of the interposition of insulating, biological layers; And also because the electrodes are considerably larger and do not target a precise fiber, but many fibers originating from different bundles, some of them having possibly different directions or polarity.

Preliminary measurements were performed using 9 volunteer subjects (13 eardrums : JMB-L, BB-L, JC-R, LD-L, JB-L, JB-R, BA-L, NS-R, NS-L, AS-R, AS-L, MR-R, MR-L). All subjects were alert and in good health. We measured the **synchronous tympanic potential** (termed *piezotympanic*) for each subject from approximate directions F, H, A or G (**Figure 1a**) and at different frequencies for at least one ear.

Our measurements show only trends or ratios but statistical analysis (SOM) reveals that there are a few factors, which are the sources of the observed variations (fibers length, surface and contact resistance, age of the subject, real sound intensity level near the eardrum).



(Figure 1a)



(Figure 1b)

(Right tympanic membrane as seen through a speculum) This image is a derivative schema of Gray909.png.

The following letters were added by us

A or G: "radii" types of fibers of collagen ;

HH' : arbitrary cord joining two peripheral points

F : manubrium of the malleus

GAH'H : annulus fibrosus (green)

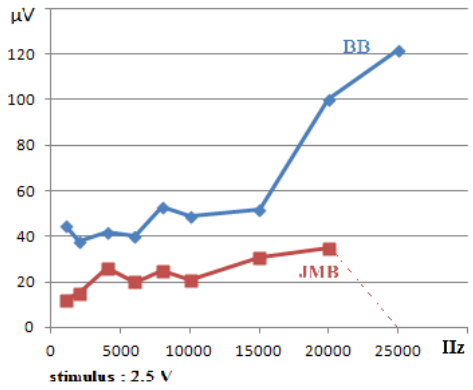
In order to verify the piezoelectric activity of the tympanum, an electrode is placed on the manubrium, another on the periphery according to the straight lines A or G.

In order to evaluate the electrical behavior of points belonging to the central structure (manubrium) during acoustic stimulations, electrodes can be placed at two points on the same side of the manubrium (F). This system can detect whether there is an electrical isochronism between these points (synchronous potential close to zero). On the contrary, electrodes can be placed facing each other on either side of the manubrium. This latter system will allow us to capture the activity of a bundle of circular fibers.

In order to evaluate whether the points belonging to the external part of the annulus fibrosus generate notable differences of potential among themselves, electrodes are placed at intersections between Line HH' and the most external part of the annulus fibrosus (GAH'H).

[4] Material and Methods are available as supporting material on Science Online – section A.

As an example, here are responses measured on two men:



Synchronous Potentials vs acoustical frequencies
Sujets : JMB, 51 ans et BB, 17 ans.
(figure 2)

Mesurements of the piezo-electricity of animal subjects

A number of animal subjects such as cats, or dogs ([81, 82]) were rejected because of the great length and severe angles (90% in the case of dogs) of the External Auditory Canal, EAC (See SOM : B : Measures on anesthetized animals, dog and cat). The EAC of chinchillas is not severely angled and their tympanic membrane is rather large. So we chose to take electrical measures, using video endoscopy (Optomed endoscope), on two chinchillas, pre-anesthetized with medetomidine (Table 2 and Figure 3

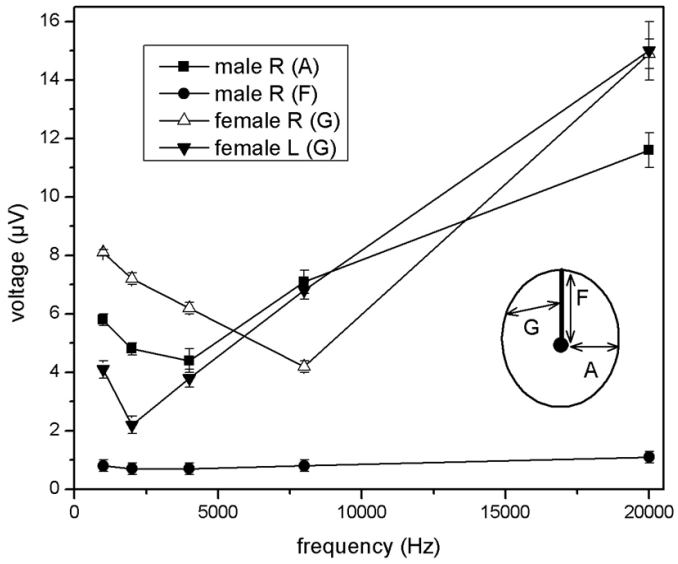


Figure 3

Synchronous potentials (µV) measured on the tympanum of 2 chinchillas (pre-anesthetized with medetomidine) : Voltage in function of acoustic frequency (log Hz). The letters A, F and G are directions of measure, identified according to the same criteria as in humans (insert). The Right Ear is noted *RE* and Left Ear *LE*.

Taken together, these measures on both human and animal subjects show that the tympanum responds to acoustic stimulation by a synchronous electrical potential. We attribute this synchronous potential to its collagenous fibers. This result corresponds to the outcomes of several studies with respect to collagen in vivo ([70, 83, 84, 85]).

The literature has shown that a residual potential (about 10% of the cochlear *microphonic* potential) persists in cases where the OHCs are destroyed or no longer function for whatever reason, and this persistence cannot be attributed either to the OHCs or to the IHCs ([86,] Cf. SOM). In addition, the destruction of the IHCs (chinchilla) does not alter the cochlear microphonic and does not alleviate the Electrically Evoked Oto Acoustic Emissions (EEOAE); Moreover, these responses tend to increase at high frequencies ([87]). That being the case, we suggest that the residual potential is due to the piezoelectricity of the tympanum.

It is generally accepted in the literature that synchronous potentials recorded at the level of the mastoid are purely of cochlear origin. If this were the case, obstructing the external ear canal should lead to a reduction of these synchronous potentials. We have been able to record a synchronous potential from the region of the mastoid bone which is not weakened when the auditory canal is occluded.
Similarly, if a sound is sent to the eardrum and not to the superficial mastoid, synchronous potentials are lower than if the sound is sent to the superficial mastoid and not to the eardrum (see SOM text). Therefore, we demonstrate that if a sound is sent in the direction of the mastoid area, the synchronous evoked potential is not from the cochlea, but from local generators. This means that neither the ossicular chain nor the Traveling Wave (TW) is involved. Rather, the synchronous potential is attributable to the collagen present in the mastoid region.

In order to build a tympanum restricted to its mechanical effects, the evolutionary process should have led to the generation of collagen fibers in a convenient geometrical arrangement, but with random polarity.

Statistics ([88])

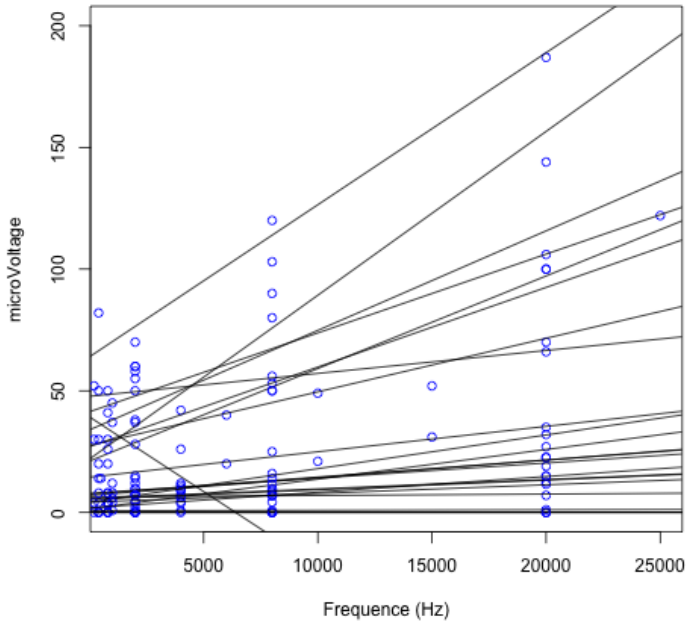


Figure 4 : Linear regression models on the tympanum series
Synchronous potentials measured at several frequencies and several directions on (either one or two) eardrums of nine human subjects : each line represents one of the directions for one of the subjects

Contrary to what might be expected, the synchronous tympanic potential (pT) is dependent upon frequency in such a way that the voltage increases along with acoustical frequency for every subset of measures. All but one of the lines representing the regression models for each row (series) shows an increase (fig.4).

f (Hz)	LD-RE (HH') (µV)	JB LE (HH') (µV)	JB RE (***HH'***) (µV)	BA LE (HH') (µV)
200	-	-	30	
400	-	-	30	-
800	-	-	50	-
2000	-	-	60	-
8000	-	-	80	-
20000	-	-	100	-

Table 2

The synchronous electrical response between two points of the annulus fibrosus (HH') is generally impossible to measure. Regarding the JB RE exception, it might be that electrodes had been positioned, not on the annulus fibrosus, but on an inner circumferential collagen fiber. The same difficulty could be found along the manubrium of the malleus; yet it is easier to position the electrodes at the boundary of the manubrium.

The transmission of the Piezo-Tympanic electrical tension (pT) to the Deiters-Cells/Outer Hair Cell complex (DOHC)

Electrophony

Alternating electrical currents impressed either on the tympanum or on the round window, or impressed across the cochlear duct, stimulate the OHCs ([89]) and permit hearing (electrophony)[5]. Quadratic distortions that may be observed disappear for frequencies above 5 kHz ([90]). As early as 1984, in countercurrent to the mainstream current of hearing physiology, Georges Offutt proposed an "electromodel of the auditory system.(1984, GoLo Press, 191 pp.)

General observations concerning gap junctions (GJs)

"*Gap Junctions (GJs) are cytoplasmic conduits possessing large pore size (10–15 Å) and allowing communication between the intracellular milieus of two contiguous cells and the passage of small metabolites and signaling molecules (<1–1.2 kDa) between cells*" ([91]). They are composed of two connex**o**ns, or hemichannels, each of which is made up of six connexins (Cx) ([92]). The GJs facilitate chemical, and especially electrical, transmissions that can be either bidirectional or unidirectional ([93, 94, 95, 96]). The transmission of a signal by means of these « *electrical synapses* » is not dependent upon a certain threshold ([97, 98]). Further, such transmission is extremely rapid and takes place without diffusion (leakage) into extracellular spaces ([98, 99, 100]). One of the neuronal functions of GJs is thought to be synchronization between brain cells ([101-102]).

[5] Amplitude du courant utilisé < 500µA

It is noteworthy that the channels of the GJs constitute "*junctional plates*" ([103, 104]) that combine hundreds of GJs. These junctional plates allow a great increase of conductivity at the level of the junction. It is noteworthy that AC voltages (eg pT voltage) cause no net movement into the conductive medium, regardless of its length, since the charge carriers oscillate back and forth in response to an alternating electric field.

From collagen to the osteocytes and the spiral ligament

The cell bodies of osteocytes act as mechanosensors of bones ([105]). They interact with the extracellular environment by means of the Cx43 hemichannels ([106]). The osteocytes of the petrosal bone merge to form a syncytium (based on the Cx43) capable of conveying electrical signals ([107, 108, 109, 110]). The electrical transmission between osseous cells always travels in the same direction: from the interior of the bone toward its surface ([111, 112]). Electrical signals arising in the piezo-electricity of the tympanum can, thus, be transmitted to the external wall of the cochlea (spiral ligament, which is a periosteum structure[6]) via the syncytium of the sub-periostic cells. A critical relationship may be established between the mutant Cx43 proteins and non-syndromic deafness ([113, 114, 115]). The Cx43 interacts with the Cx26 of the cochlea ([116, 117, 118,119, 120, 121]), and possibly with the Cx30 ([122]) which makes it capable of transmitting the piezotympanic signal to the cochlear Deiters cells (DCs).

Role of Cochlear Gap Junctions

Genetic alterations of Cx26, Cx30, Cx30.2, Cx30.3, Cx31, Cx 31.1, Cx31.9, Cx32, and Cx43 connexins result in non-syndromic deafness ([123, 124, 125]), and the purely metabolic explanation of their usefulness (for example, the transport of K^+) seems insufficient to explain why this is so. Cx26 and Cx30 are reduced threefold from the cochlear apex to base {mainly in the DCs : [S126]}, but this finding does not weaken the hypothesis that these GJs play an essential role for all frequencies: Either mutations ([S12]) or a blocking ([S127]) of Connexin 26 GJ, produces a reduced or absent distortion product of otoacoustic emission and hearing loss at all frequencies.

The Syncitia of the cochlear Gap Junctions

There are two independent syncytia in the cochlea, and this is due to the presence of GJs.

a The GJ system of connective tissue cells

consists primarily of fibrocytes ([128, 129, 130]). The deterioration of this system results in a progressive hypoacusis, especially with respect to high frequency sounds ([131, 132, 133]). It should be noted as well that most of the cochlear fibrocytes contain a canalicular reticulum that enables the K+ ions to travel through the network that they form ([134]). The Fibroblast Growth Factors (FGFs), which regulate the electrical excitability of cells, appear to have a role in the maintenance of normal auditory function, even though this role is poorly understood ([135]).

b The GJ system of epithelial cells

is composed of root cells within the spiral ligament linked to several types of supporting cells ([136]). These enable the liaison of the cochlea with the *stria vascularis*, considered to be not only the battery of the cochlea but also its heart and lungs ([100, 137 , 138, 139, 140,141]). This epithelial cell GJ system is indispensable to audition at the cochlear level. It is noteworthy that an electric sinusoidal wave can travel along an electrolytic pathway going through the GJs with minute displacements of ions between adjacent cells but without global displacements from the first cell to the last one and back. So the epithelial cell GJ system is capable of

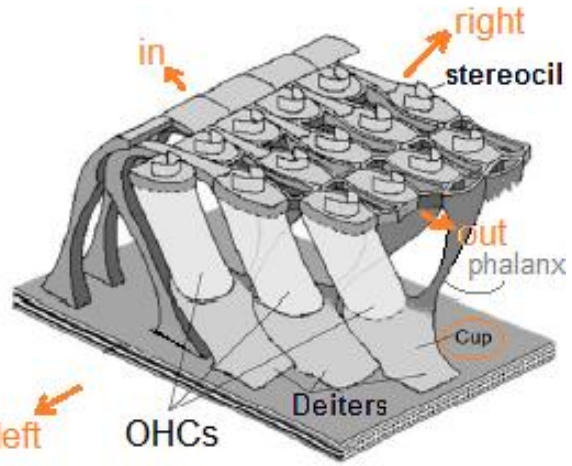
[6] See https://histo.life.illinois.edu/histo/atlas/image_js.php?sname=w82a&iname=40c1

transmitting variations of potential ([142]) from the root cells to the DCs, and, when it does not function, the OHCs, even if they are normal, lose their effectiveness ([143, 144])[7]. Thus, active cochlear amplification is dependent on the gap junctions of supporting cells ([145]).

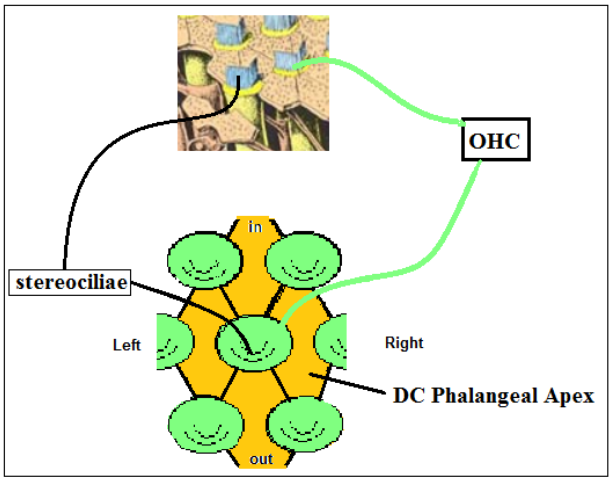
The DOHC Complex in the literature (Le complexe DOHC dans la littérature)

Definition of the DOHC Complex, alias "DOHC"

Each OHC(fig. 5 and 6 below) is surrounded by five DCs: its base is supported by the cupular body of a DC (DC_5) and its ciliated apex is bordered by four phalangeal apexes from four other $DCi_{(1..4)}s$: on the right (DC_1 or DC_r), inner (DC_2 or DC_i), on the left (DC_3 or DC_l), outer (DC_4 or DC_o); each of them being different from the DC_5.



(modified after Lagostena et al., 2001 ; see also Nam J.H.[146-147] 2014) Fig. 5

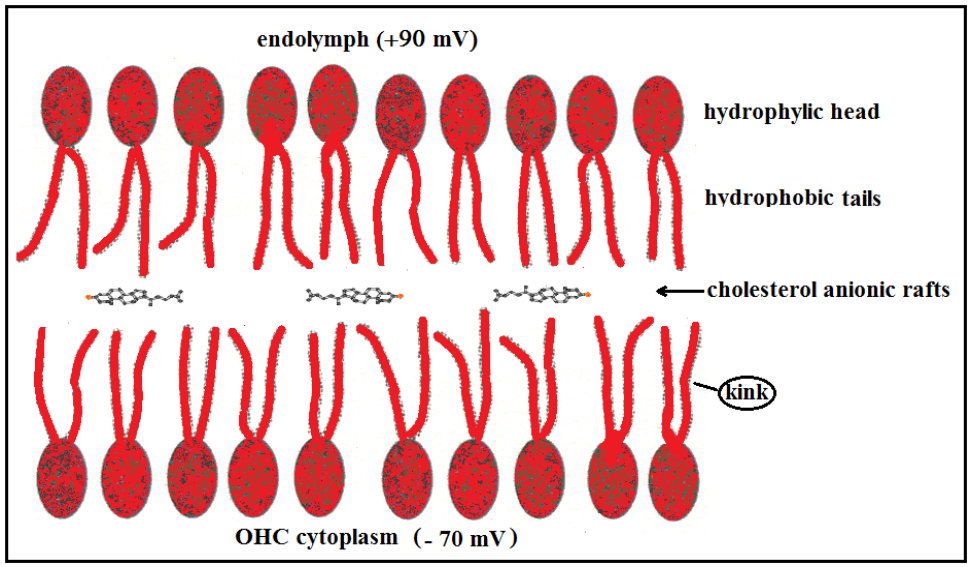


schematic top-view of the DOHC complex
Fig. 6

Stereociliae are implanted on the apex membrane of OHCs (cuticular plate). They are bathed in endolymph and mobilized by movements of the endolymph (including the TW).
We will use the acronym DOHC (standing for “Deiters cell/Outer Hair Cell”) or “DOHC complex”.

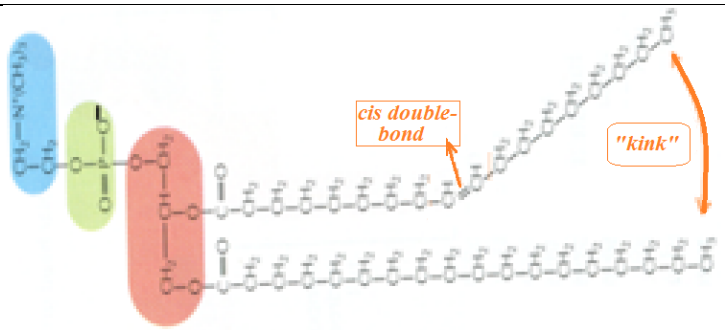
[7] It is conceivable that there might be other ways for the tympanic voltage changes to reach the cells of Deiters. For example, one could investigate whether there is a form of "conductive collagenous continuity" leading to the "stripe" of implementation of the feet of the lower limbs of the Deiters. In this case, the electrical contact would be made by the "smooth area" of the "footplate" (actin) or by its "rough area" (microtubules). It is more likely that variation in voltage through the GJs lead, on the one hand to the Cup, on the other hand to the apex of the Phalanx.

The cuticular plate bilayer ***(****La bicouche de la plaque cuticulaire****)*** (Cf Fig DOHC-4)



Lipid bilayer
Fig. 7

Like all cellular membranes[148], the cuticular membrane consists mainly of amphiphilic lipids (generally phospholipids). They have one head group that is hydrophilic ('polar') and two hydrocarbon tails that are lipophylic ('non polar'). One tail typically has one or more cis-double bonds (that is, it is unsaturated), while the other tail does not (that is, it is saturated). Each cis-double bond creates a small kink in the tail[149] (fig.7). By forming a double layer, with the polar ends pointing outwards and the non polar ends pointing inwards, membrane lipids form a 'lipid bilayer' which keeps the watery interior of the cell separate from the watery exterior[150].



(after Bruce et al. p.619)
Fig . 8

The "lipid bilayer" plays a dual role in the life of the cell: both as insulator and filter[8].
- Its insulating lipid molecules, arranged in a 5 to 10 nm thick bilayer, form an impermeable barrier to the passage of most water soluble molecules. They block the passage of inorganic ions (K +, Na +, Cl-, Ca2+,...) and hinder the diffusion of polar organic solutes such as amino acids.

[8] It is a place of selective Exchange between the inside and the outside of the cell (Michel Mitov Sensitive Matter - Foams, Gels, Liquid Crystals, and Other Miracles, Harvard University Press, 2012, Harvard Univ. Press 2012).

- It is a filter as well: protein molecules regulate transmembrane exchanges; in itself, the membrane is permeable only to small hydrophobic molecules (O2, N2, glycerol,...).

The hydrophilic surface of the outer leaflet of the cuticular membrane is loosely associated ("diffuse layer") to a layer of positive ions (+90 mV) of the endolymphatic aqueous space. The hydrophilic surface of the inner leaflet of the membrane is loosely associated ("diffuse layer") to negative ions (-70 mV) of the intracellular aqueous space. So the lipid bilayer is associated with an electronic double layer which has a non-linear capacitance dependent on the voltage applied.

The lipid bilayer is a lyotropic liquid crystal

The structure of the phospholipid bilayer is a liquid crystal (of smectic type). An electric field applied to a liquid crystal, typically found in a flat screen, changes the orientation of the dipole molecules of the liquid crystal. If the molecules are initially not permanent dipoles, they can, nevertheless, become induced (by the field) dipoles, whose orientation persists in the presence of the field.

The molecules of the bilayer are "stirred" when they are stimulated by a variable electric field. However, they are forced to maintain their insertion between the sister-molecules that are parallel to them[151]..
Meanwhile, the cholesterol can become aligned in the direction of the electric field emanating from the phalanxes. As this electric field varies depending on the evolution of the pT, cholesterol is mobilized according to the same rhythm ([152, 153]) - and its movements could act upon the opening of ion channels.

Phosholipids of the bilayer are very important for OHC function

The leaflets of the bilayer are mainly composed of phospholipids, and anti-phospholipids can negatively affect hearing. Chlorpromazine, which intercalates into the inner leaflet of the phospholipid bilayers, alters OHC electromotility without a known direct action on prestin [[154, 155]]. So, the intervention of the **phospholipids** concerns neither the action of the stereociliae, nor that of the prestin but rather that of the reticular lamina.

Ionic Channels in the literature (Canaux ioniques)

Poration and preporation

The exposure of a lipidic bilayer to an electric field exceeding 150 mV, leads to variations of its conductance, due to the creation of lipid pores (*electro-poration*). This phenomenon increases with duration of exposure[156].
These pores emerge "at random" on the basis of a metastable state of the membrane called "preporation",. (i.e. lipid reorganization, resulting in transient increases in the permeability of the membrane to ions).
The properties of a pore are determined by the structure of its prepore. Prepore and pore can be considered as two sub-states of a common structure[157, 158].

Interestingly, the Outer Hair Cell cuticular bilayer is permanently subjected to an electric field of about 150 mV (+ 90 mV in the endolymphatic fluid and -70 mV in the intracellular environment). Thus, one may suggest that the cuticular bilayer is in a continuous state of 'preporation' and that variations of the intramembranous electric field can open the prepores in a field-sensitive way.

Description of the cuticular Ionic channels (Description des canaux ioniques cuticulaires)

The lipidic prepores and pores have characteristics very similar to certain protein ion channels ([159, 160]).
which are proteins forming a selective **pore**[161] in the cell membrane.

They exhibit two essential biophysical properties: a high permeation rate coupled with a high ionic selectivity, without any energy supply. They can be characterized by conducting (open) and non conducting (closed) conformations activated by specific stimuli[162].
Many **ion channels** may be created and link the endolymphatic aqueous medium to the intra-cellular aqueous medium through the lipid bilayer.
In most cases, the gate opens in response to a specific stimulus. The main types of stimuli that are known to cause ion channels to open are a change in the voltage across the membrane (voltage-gated channels) or the binding of a ligand (ligand-gated channels).

The working of cuticular ionic channels seems to be correlated to the lipid rafts ([163, 164, 165, 166]).
Intramembranous ionic cholesterol not only modifies the voltage across the membrane (from the internal space between the two leaflets of the bilayer) but also seems able to bind with selective ionic-channel-proteins and to modulate their function ([167, 168, 169]).

Effect of quantitative variations of the cholesterol membrane of OHCs

Cholesterol is a major component of cell membranes and constitutes up to 50% of lipids in membrane rafts[170].
Membrane cholesterol is involved in signal transduction by affecting the activity of protein receptors to which it preferentially associates[171].
There is a dynamic and reversible relationship between membrane cholesterol levels and voltage dependence of prestin-associated charge movement in OHCs[172, 173].

Poly Unsaturated Fatty Acids (PUFAs) and cholesterol mobility inside the cuticular plate

Membrane proteins modulate excitation in certain receptor cells, and PUFAs contribute to the coding of auditory stimuli via the arachidonic acid[9]-sensitive potassium channel in the cochlea[174].

Exposure to noise generates "reactive oxygen species" (ROS[10]), which attack the PUFAs of cochlear cell membranes, resulting in deafness[175]. Thus, antioxidants protect the PUFAs and, through the administration of n-acetyl-cysteine[176], an improvement of about 20 dB can be observed on a guinea pig subjected to excessive noise. PUFAs are preventive and curative of the presbyacusis ([177, 178]).
People with Usher type I have lower levels of long-chain PUFAs than control subjects[179]; they are profoundly deaf (anacusis).

The findings from the neutron diffraction work reported below (see fig.10) suggest that the cholesterol molecule stays at the center of the bilayer and is constrained to a maximum displacement inferior to 6 Å relative to the cellular body as a whole ([180]).

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[9] Essential fatty acid

[10] http://en.wikipedia.org/wiki/Reactive_oxygen_species; see also oxidative stress. "***Reactive oxygen species*** *(ROS) are chemically reactive molecules containing oxygen. Examples include oxygen ions and peroxides. ROS are formed as a natural byproduct of the normal metabolism of oxygen and have important roles in cell signaling and homeostasis. However, during times of environmental stress (e.g., UV or heat exposure), ROS levels can increase dramatically. This may result in significant damage to cell structures. Cumulatively, this is known as oxidative stress. ROS are also generated by exogenous sources such as ionizing radiation*." (after wp, 2014 – 12 – 03).

Cholesterol has a poor affinity for phospholipids that contain PUFAs (e.g. arachidonic acid[11]).
The sterols show a strong preference for embedding themselves between bilayer leaflets, which is an unusual placement for them[181].

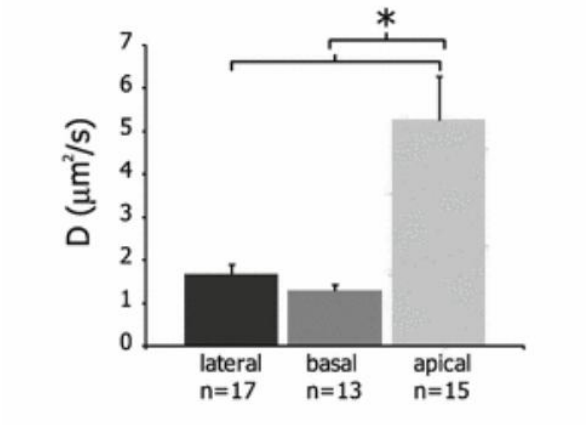

Fig. 9

Effective diffusion coefficients, *D* (μm /s), across the three OHC regions. D values in the apical region were significantly ($p<0.0005$) larger than in lateral or basal regions[182].

There are quantitative differences in lipid lateral mobility[12] of cholesterol, and rafts, among the apical, lateral, and basal regions of the OHC[183] (fig.9). The great diffusibility of cholesterol and lipid rafts at the level of the cuticular plate suggests that their mobilization plays an important role at the level of the DOHC complex[184].

Not only does cholesterol have an affinity for saturated hydrocarbon chains, but it also has an aversion to PUFAs[185]. In high PUFA content bilayers cholesterol becomes very mobilizable and, simultaneously, capable of assuming different orientations within a bilayer[186]. We can, thus, infer that the cuticular membrane contains a large quantity of PUFAs. This should be verified experimentally.

Effects of movements of the polarized cholesterol onto the opening of ion channels

An important physical effect of cholesterol, and its derivatives[187], is the modification of the membrane's internal electrical dipole potential ([13]), ψ_d: This effect is one of the major mechanisms by which it modulates ion permeability ([188, 189]) and the molecule-membrane interactions in lipid rafts with possible effects on cell signaling ([190, 191]).

Variations of membrane potential of the phalangeal apexes affect the lipid rafts of the cuticle of the OHC, thus causing shifts in the lipid bilayer. These can be shifts in the plane of the membrane and/or changes of orientation of the molecule axis (defined as the direction of the C-OH link, which connects the sterol ring

[11] Arachidonic acid is a polyunsaturated fatty acid present in the phospholipids (especially phosphatidylethanolamine, phosphatidylcholine, and phosphatidylinositides) of membranes of the body's cells, and is abundant in the brain, muscles, and liver (en-WP, 2014 12 04).
[12] Qantifying the lateral mobility (translational diffusion) of molecules in the plane of the bilayer is a useful method for directly measuring membrane fluidity and for indirectly assessing changes in other interconnected material properties (e.g. electrostatics, hydrophobic thickness, etc.) of membranes in live, native specimens.
[13] The dipole potential is an **electrical potential within lipid membranes**, which arises because of the alignment of **dipolar residues of the lipids and/or water dipoles** in the region between the aqueous phases and the positive hydrocarbon-like interior of the membrane. Depending on the structure of the lipid, its magnitude can vary from ~100 to >400 mV.

system to the –OH end). In both cases, these movements are likely to influence the degree of opening or closing of ion channels[192].

Ions relevant at the level of the OHCs ionic channels are **calcium,** potassium and chlorine ([193]). It has been shown that Ca2+ currents could drive the active process of the cochlea ([194]).

A very fast time resolution is necessary to achieve the higher frequencies heard by some mammals (200 kHz) [195, 196], ie 200 x 10^3 compressions and 200 x 10^3 rarefactions per second acting on the eardrum[197].
Prestin alternately shortens and lengthens movements isynchronous to these compressions and rarefactions , in such a way that 400x10^3 movements are required for amplification with a correct tuning of the tympanic sound by prestin.
Ionic biochannels show ion selectivity, permitting only ions of an appropriate size and charge to pass, but not others; The ions often pass in single file[198].
Known fluxes of ion channels in biological systems (up to 10^8 ions per second) would indeed allow a sampling three orders of magnitude higher than the Nyquist criterion. This is especially the case as the information is conveyed, not by one, but by many channels ([199]).

The cholesterol acceptor of the phalangeal electric field

The cuticular bilayer has a system of cholesterol rafts between its two leaflets. These cholesterol rafts modify the passage of electrons in certain directions[14, 200]. Thus we think that a "grid" is constituted, favoring or discriminating against the passage of holes (cations), depending upon their direction with respect to the bilayer plane.

Cholesterol and PUFAs

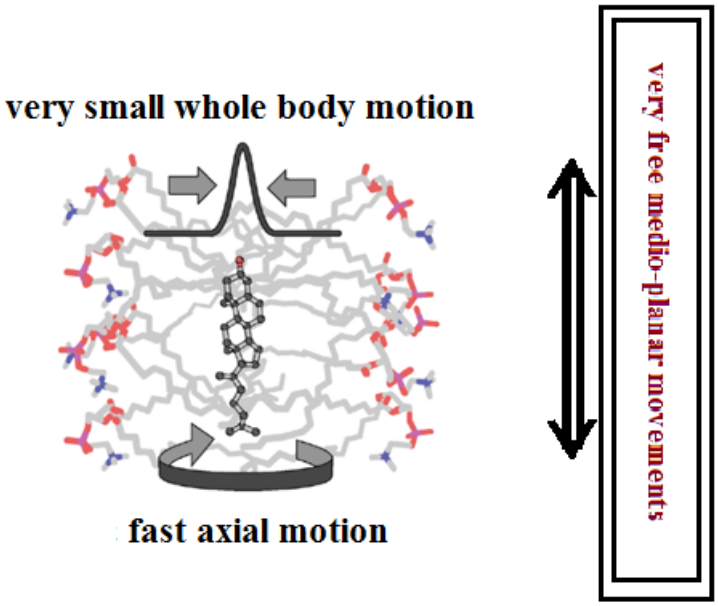

fig. 10

Schematic depiction of the location of cholesterol in “20:4-20:4PC” membranes. The molecule is found by neutron diffraction to reside at the center of the bilayer and to be motionally constrained to ± 6 Å of whole body motion. At the same time, the molecule also undergoes fast axial rotation, as determined by D NMR spectra ([201]).

[14] "*The membrane dipole potential spatially varies about the cell surface, particularly within membrane micro domains such as 'rafts'* " O’Shea, 2003

If the rate of PUFAs is low, the hydroxyl group of anionic cholesterol is located near the lipid/water interface ('*upright*' orientation). If the rate of PUFAs is high, cholesterol is able to simultaneously adopt different orientations within a bilayer, and it prefers to remain confined between the two leaflets and parallel to them ('*flat*' orientation)[202, 203].

Does the cuticular membrane have semiconductor properties?

The orbital overlap of two π-systems separated by a non-conjugating group, such as CH2 (Homoconjugatio[15] ; IUPAC, 1994) may endow a biological molecule with semiconductor properties[16]. When biological PUFAs have several double bonds separated by a saturated bond, they have the properties of a semiconductor.

The DOHC intercellular pathway

In physiological conditions, however, lipids do not cross TJs[204, 205]: There is a strict insulation, chemical as well as electrical, between the two bound cells, and no current will flow from one to the other of their apical membranes [206, 17]. For this reason, no electric current can cross the barrier between the phalangeal apexes of the DCs and the cuticle of the embedded OHC ([207]).
However, this border cannot prevent a hydrophobic intercellular electrostatic coupling unrelated to any GJ; The capacitance of a cell membrane varies according to the voltage applied to the membrane of a neighbouring cell ([208]). If we apply this model to the DOHC complex, we can suggest that the capacitance of the cuticular bilayer varies in a manner controlled by the voltage of the contiguous phalangeal apexes. In this way, the piezoelectric information, carried from the tympanum to the four phalanxes of the DCs, should cause its capacitance to vary in a synchronous manner.

Mobilization of the intramembranous ionized cholesterol by variations of phalangeal origin in the electric field .

If the PUFAs rate is sufficiently large, the polarized molecule of cholesterol moves freely in the median plane of the bilayer. It can, thus, be subjected to centripetal or centrifugal movements from a center represented by the intersection of the apico-basal axis of the OHC and the median plane of the bilayer.
The differences in voltage between adjacent membranes mobilize, electrophoretically, the intra-membrane anionic cholesterol (OH^- ends). When phalangeal apexes are the seat of a positive half-wave, particles of cholesterol anions are attracted towards the periphery of the OHC cuticle; on the other hand, if the phalangeal apexes carry a negative half wave, particles of cholesterol anions are pushed back towards the center of the OHC cuticle.

Electrical as well as mechanical stimulation of the DCs can modulate the electromotility of the OHCs.

Cx26 expression in the cochlear supporting cells plays a critical role in active cochlear amplification[209] and its targeted deletion can eliminate active cochlear amplification[210, 211]. It is coincident with a large reduction in

[15] In the original meaning a conjugated system is a molecular entity whose structure may be represented as a system of alternating single and multiple bonds: e.g. CH_2=CH–CH=CH_2. In such systems, conjugation is the interaction of one p-orbital with another across an intervening σ-bond.
[16] A common feature of organic-FET materials is the inclusion of a conjugated π-electron system which serves as the active semiconducting layer, facilitating the delocalization of orbital wavefunctions.
[17] Nevertheless, a large enough voltage difference could overcome what is normally an impenetrable energy barrier (Turin, 1991).

distortion product otoacoustic emission (DPOAE[212]) and severe hearing loss at high frequencies (changes are greater in the shortest OHCs)[213].
Stimulation of the DCs, either electric or mechanical, can modulate the electromotility of the OHCs[214] and in vivo active cochlear amplification depends on GJs between the DCs[215].
The separation of a DC phalanx from a related OHC cuticle, or of a cupule from the OHC that it supports (or the elimination of GJs between the bodies of Deiters cells) results in deterioration of the electrical behavior of the concerned OHCs ([18]).

There being no electrical synapse (GJ) between DCs and OHCs, it has been proposed that this electric effect on the OHC is due not to an electrical interruption between DCs and OHCs, but rather to an interruption of a purely mechanical nature ([19]).

The destruction of the cytoskeleton annihilates the electric effect of the DCs on the OHCs

The apex of the phalanges of the DCs is connected to the cuticular plate of OHCs by Tight-Adherens Junctions (TAJs). These TAJs bring into contact the cytoskeleton of adjacent cells ([216]) (Fig. 5). Microtubules and actin filaments of the cytoskeleton are capable of transmitting electrical signals ([217, 218, 219, 220, 221, 222]) and are comparable to a RCL circuit, having resistance, non-linear capacitance and inductance ([223, 224, 225, 226, 227, 228, 229, 230]). Both are present, especially in the apical region of the cytoskeleton, forming part of the cuticular plate and reticular lamina ([231]). They can transmit and amplify electric signals via the flow of condensed ion clouds ([232, 233, 234, 235, 20]).
Furthermore, the destruction of the cytoskeleton of the DCs negates the effect of electric stimulations of the DCs on OHC electromotility[236]. This implies that the cytoskeleton of DCs plays a critical role in the modulation of OHC electromotility. According to Yu and Zhao (2009) this is evidence that, if electrical stimulations of DCs influence OHC electromotility, it must be through the DC-OHC mechanical coupling rather than by extracellular field effect[21].

However, the electric voltage of the phalangeal apex necessarily causes variations of the electric field in the OHC cuticle (via the intercellular border at the level of the TJ). Indeed, the destruction of the phalangeal cytoskeleton removes the electrical activity of the phalangeal cytoskeleton and, as a consequence, its field effects.
The measurements used by Yu and Zhao were taken using the whole-cell patch clamp method, i.e. with electrodes implanted into the cytoplasm of the DC and the OHC, but not within their respective bilayers; Thus, weak capacitive interactions, consistent with the action of an FET grid, escape measurement[22].

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[18] Yu N, Zhao HB. Modulation of outer hair cell electromotility by cochlear supporting cells and gap junctions. PLoS One. 2009 ;4:e7923. [PMC free article] [PubMed]
[19] Yu N, Zhao HB. Modulation of outer hair cell electromotility by cochlear supporting cells and gap junctions. PLoS One. 2009 ;4:e7923. [PMC free article] [PubMed]
[20] According to Szarama (pers.com., 2012) it would be interesting to design experiments for the purpose of distinguishing the effect of ionic concentration changes from the effect of the mechanical resistance of the cytoskeleton. Such experiments would serve as a starting point for a better understandingof the paths of signal transduction in which microtubules are involved.
[21] A question arises as to the method of separation between DCs and OHCs: in the photos shown, it seems that the same DC supports an OHC and is connected by its own phalanx to the same OHC. This is surprising since several concurring works assert that each OHC is bound by TJs with four DCs, all different from the fifth DC that serves as support for this same OHC (cf. § The DOHC Complex in the literature).
[22] Is there a field effect phenomenon limited to the TJ, and involving, on the one hand, the cytoskeleton of the DC and, on the other hand, the {anionic cholesterol + ion channels in the membrane of the OHC}, this field-effect does not necessarily occur between cytoplasmic spaces.

Cupular phenomena represent a more sensitive issue. The {DC Cup /OHC base} junction is very unusual (see fig. 11 - 12) : at the level of the cupule it includes hemichannels, whose activity is confined in the cupular slot; It is an extremely small dedicated space, without obvious communication with what is generally regarded as the extracellular medium or the Nuel space.

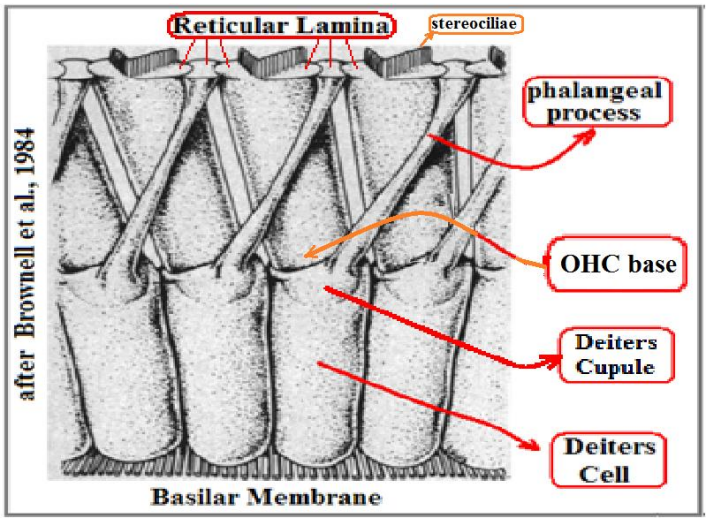
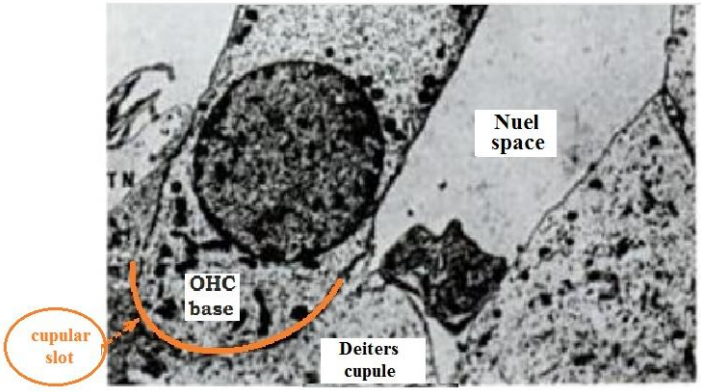


Fig. 11



Adapted from Roger Coujard, Jacques Poirier (1980),
Précis d'histologie humaine – p. 728

Fig. 12

Yu and Zhao ([23]) have put the extracellular milieu into communication with the earth, probably allowing effective control of the extra cellular environment after the breakdown of the cupule-OHC junction. This control is irrelevant, however, as regards variations affecting the slot between the cupule and the OHC as a specific space. The same is true for the variations into the intra-membranous TAJ cuticle-phalanx space.

In another experiment, these authors blocked the ionic channels of the OHC basal membrane in such a way that the ionic changes in the cupular slot could not elicit charge-carrier exchanges between OHC and cupule. But, of course, when the physical joining between the DC cup and the OHC base is preserved, this precaution does not

[23]Yu N, Zhao H-B (2009) Modulation of Outer Hair Cell Electromotility by Cochlear Supporting Cells and Gap Junctions. PLoS ONE 4(11): e7923; doi:10.1371/journal.pone.0007923

prevent the intervention of a capacitive electrical signal between cupular and OHC membranes. So, the blocking of ion channels is not sufficient to eliminate all of the effect of the Deiters cupule in the OHC membrane.

The OHC and the cupule are joined at the membrane level. The two membranes act as capacitors, and mechanically severing the coupling between them, as was done in this experiment, changes the capacitance of the set radically. In the case of the separation between cupule and OHC, the cupular slot enclosure is totally open and allows a fast diffusion of ions from the cupular hemichannels: it follows that ionic changes from the cupule get "lost" in the extracellular medium and cannot reach a level sufficient to act upon the OHC.

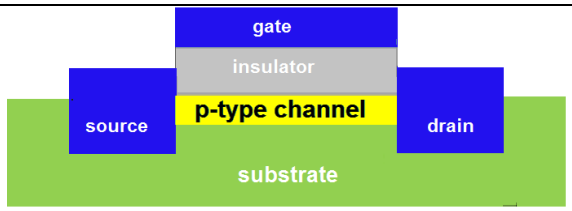
A field effect into the DOHC complex ?

We believe that there is an electrostatic link (a field effect) between the cytoskeleton apex of the Deiters phalanxes and the OHC cuticle. This link exists without a significant current, and so without electrical conduction by gap-junctions. It is clear that this field effect must be detected mainly by the effects it produces within the cuticular bilayer, where it likely acts as a grid on the communication between the set {stereociliae / endolymph} as the source and the set {OHC cytoplasm, prestin, baso-cellular membrane, cupular slot} as the drain (cf. § A field effect into the DOHC complex ?.

According to Yu and Zhao (2009)[24], the electric effect of DCs on an OHC must be assigned to an intermediate phenomenon of a mechanical nature. But we believe that there is also an electrostatic effect. Indeed, the field effects in the DOHC suggest that it could operate in a way similar to a triode (like an FET).

Definition of a field effect transistor (FET)

A Field Effect Transistor[25] (FET) uses an electric field applied by a grid (gate), to control the conductivity of a "channel" (in yellow on the diagram, fig. 13) in semiconductor materials.



p-type FET
fig. 13

Field effect between phalangeal apexes and anions of cholesterol

We offer a different interpretation of the phenomenon highlighted by Yu and Zhao. The depolarization of the DCs has an effect on non-linear capacitance and distortion products related to the electromotility of the OHCs[237]. The phalangeal voltage variations of the DCs cause changes in the electric field of the int**ra**membranous anionic cholesterol, which is mobilized by electrophoretic effect[238].

[24] Yu N, Zhao HB. Modulation of outer hair cell electromotility by cochlear supporting cells and gap junctions. PLoS One. 2009 ;4:e7923. [PMC free article] [PubMed]
[25] Lilienfeld Julius Edgar, Method and apparatus for controlling electric currents, patent US 1745175 (1930-01-28)

Les stimulations électriques aussi bien que les stimulations mécaniques des DCs peuvent moduler l'électromotilité des OHCs[239].
Both electrical and mechanical stimulations in DCs can modulate OHC electromotility[240].

The variations of the electric field produced by the coordinated electrostatic action of phalanxes cause the electrostatic field of the cuticular bilayer to vary. This effect concerns mainly the rafts of anionic cholesterol (endings OH-) of the median zone, and this leads to several consequences:

- In the median plane of the bilayer, these variations create forces of radial orientation, on the rafts of cholesterol. [cf. § The DOHC intercellular pathway].
- They involve a variation of the membrane capacitance (same reference).
- They tend to change the orientation of the rafts of anionic cholesterol, by rotating them in the median plane of the bilayer, depending upon the strength of the respective fields of four phalanxes. [cf § Effects of movements of the polarized cholesterol onto the opening of ion channels].
- They may modify the rotation of the anions of cholesterol around their own axis (in the context of double layers rich in PUFAs). [Cf. § Cholesterol and PUFAs]
- They are able to trigger alternately opening and closing of selective ion channels (Ca2 +, K+, Cl-). [cf. § Description of the cuticular Ionic channels (Description des canaux ioniques cuticulaires)].

FETs and Trickystor

We propose the name of *trickystor* for the structure linking the phalangeal apex and the cuticle of the OHC.
The *trickystor* structure, an important component of the DOHC complex, shares similarities with an FET, as is shown in the following table :

FET	Trickystor
An FET, like a triode, consists of three technological electrodes:	The trickystor consists of three organic electrodes: a source, a grid (or gate) and a drain.
1- The source	The set { stereociliae, endolymph }
2- The grid	The set {phalangeal apexes, lipido-cholesterol rafts}
3- The drain	The set {cytosol, inner aspect of prestin }

The potential of the grid is the result of an electromagnetic field,	The phalangeal apexes radiate an electromagnetic field that acts on the lipido-cholesterol rafts
This potential creates and controls the conductivity of a channel in a semiconductor material.	The voltage of the phalangeal apexes acts on the metastable poration of the bilayer. This action is made possible by the semiconductor properties associated with the PUFAs. As a result there are changes in the transmembrane conductivity.
Only one type of load carrier is conveyed in the channel	Positive ions of the endolymph are conveyed in ionic channels having selective properties.
The conductive channel connects two ohmic contacts, the source and drain	The conductive channel connects two ohmic contacts, [stereocilia and endolymph] on the one hand, and [cytosol and the internal aspect of the prestin] on the other.

The grid is strictly isolated from the channel.	The TJ, for moderate differences of potential, strictly isolates the cuticle from the phalangeal apex
The voltage applied to the grid controls the amount of charge carriers which circulate in the conductive channels.	idem

The FET Channel and Channels of the DOHC

Semiconductivity features :

1) On one hand, the cuticular bilayer of the OHC consists of liquid crystals of lipids, a large proportion of which are PUFAs. PUFAs are π-conjugated systems. They can, therefore, be considered to possess semiconductor properties.

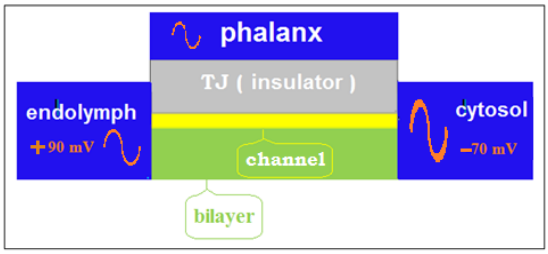
2) On the other hand, semi conductivity attributable to the metastability of ion channels in preporation state (effect of electric field on the anions of cholesterol) (cf.§ Poration and preporation).

It remains to be determined if these two mechanisms [(1) and (2)] cooperate, or are identifiable one to the other. Experimentally, the variations of voltage of the DCs bring about electrical variations in the OHCs. In our electrogenic interpretation of this phenomenon, the voltage of the phalanxes (issuing from the pT potential), applied to the rafts of cholesterol, controls the number of charge carriers circulating in the system from the source to the drain.

Destroying the cytoskeleton should result in the elimination of the electrical function of the microtubules as well as the elimination of their mechanical function with respect to the OHCs. The destruction of the cytoskeleton annihilates the electric effect of the DCs on the OHCs .
Without significant power, and so, without electrical conduction by GJs, there remains an electrostatic link between four phalanxes of Deiters and the cuticle of the OHC. Here, as in an FET, the grid current is null (or negligible) in a static regimen, since the grid behaves as a low-capacity capacitor.

Trikystor and Hearing physiology

Thus, we can schematize (fig. 14) the *trickystor*, considered as a kind of FET:



trickystor

Fig. 14

Field effect transistors are amplifiers up to very high frequencies, and they do not have the low-pass limitation problem in the accepted scheme in which the bilayer is a simple non-linear capacitor (cf le § **Erreur ! Source du renvoi introuvable.**).
Variations in potential of the source are amplified by homologous variations of the grid potential; This effect is not dependent on the frequency and consumes no power[26]; It occurs only if there is good insulation between the phalanx and the cuticular bilayer at the level of the TJ.
Thus, at the level of the DOHC, the AC (Alternating Current) that originates at the level of the eardrum (or bone) acts on the alternating current created by the ciliary movements under the influence of the TW; If the ciliary signal (resulting from the TW) and the phalangeal signal (issued from pT) are approximately in phase, the resulting signal is amplified, otherwise it is attenuated. This redundancy enables the elimination of a significant part of any noise, whether electrical or mechanical.

Synthèse

The mechanical waves carried by the TW to the stereocilia are transduced at that point into an isomorphic alternating current. This alternating current crosses the cuticular plate and is modulated by the field effect from the voltage of the phalanxes of the four nearby DCs; As we have seen, this voltage derives from the piezo-electricity of the eardrum (pT).
The structure of the DOHC complex, which suggests a kind of triode of the FET type ("*trickystor*"), amplifies the signal and optimizes the signal-to-noise ratio.
Thus, the DOHC complex can act as a selective amplifier, and redundancy eliminates the possibility of parasites disturbing the signal. Further, there is a cupular Deiters signal as well, which intervenes on the external aspect of the prestin.
If the signals from the top (stereociliar, quad-phalangeal) are in phase, and if the cupular signal is in antiphase, contractions and elongations of the prestin amplify the TW considerably and sharpen the tuning. Otherwise, the signal is greatly weakened so that multiple redundancy eliminates inappropriate interferences.

Diagrams (or graphic summaries)

Physiological Diagram of the "Covert Path"

To give an overview of our hypothesis of a "covert path", we propose the following diagram.

[26] Regarding our interest in the piezoelectric properties of the eardrum, Fabio Mammano objected that the voltages we are measuring are very weak, on the order of 10 to 200 microVolts. However, the grid voltage does not necessarily have to be of a large value in order to produce an effect.

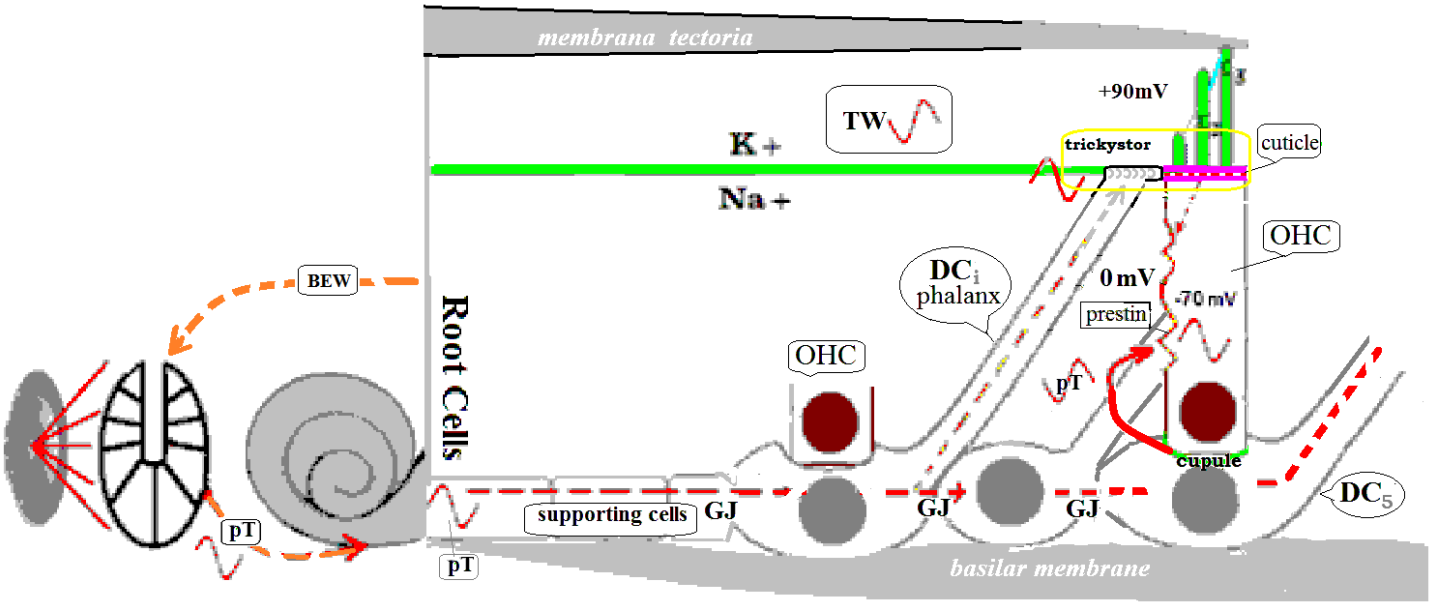


Figure 15 : Diagram of the "Covert Path"
(dotted line going from left to right)

The tympanum vibrates in response to an environmental sound. The piezoelectric collagen of the tympanum engenders an electrical signal (piezotympanic or pT). The potential of this electrical signal has the same morphology as the sound signal. The pT is carried by the GJs of the supporting cells (Cx43, Cx26, Cx30, etc.) and reaches the phalanxes of $DC_{i\,(1..4)}$s in the vicinity of a given OHC as well as the cupule of the DC_5 that supports the OHC in question.
The cytoskeleton of the phalanxes of the DC transmits voltage variations of the four $DCs_{i(1..4)}$ to the phalangeal apexes. This drives, depending upon the morphology of the pT, a synchronous variation of the field effect at the level of cholesterol rafts in intramembrane median situation. The whole constitutes a specific type of FET: a *trickystor*. If the phalangeal signal is isomorphic with the original stereociliar electrical signal, this results in an amplification of the signal. The non-isomorphic components of the signal are attenuated.

The ensuing signal reaches the intracellular aspect of the prestin. If this cuticular signal is in antiphase with the cupular signal coming from the extracellular aspect of the prestin, the prestin is mobilized, thus amplifying the signal even more. In contrast, if this cuticular signal is in phase with the cupular signal, the prestin reacts weakly and there is a damping of the signal.

Diagram of the local amplifying circuit

Below (Fig. 16) we propose the design of an *equivalent circuit*[27] that presents the classical pathway (in black) supplemented by the "covert path" (in red). This diagram should be checked for compatibility with published quantitative works (see S[241]).

[27] In electrical engineering and science, an **equivalent circuit** refers to a theoretical circuit that retains all of the electrical characteristics of a given circuit. Cf. http://en.wikipedia.org/wiki/Equivalent_circuit

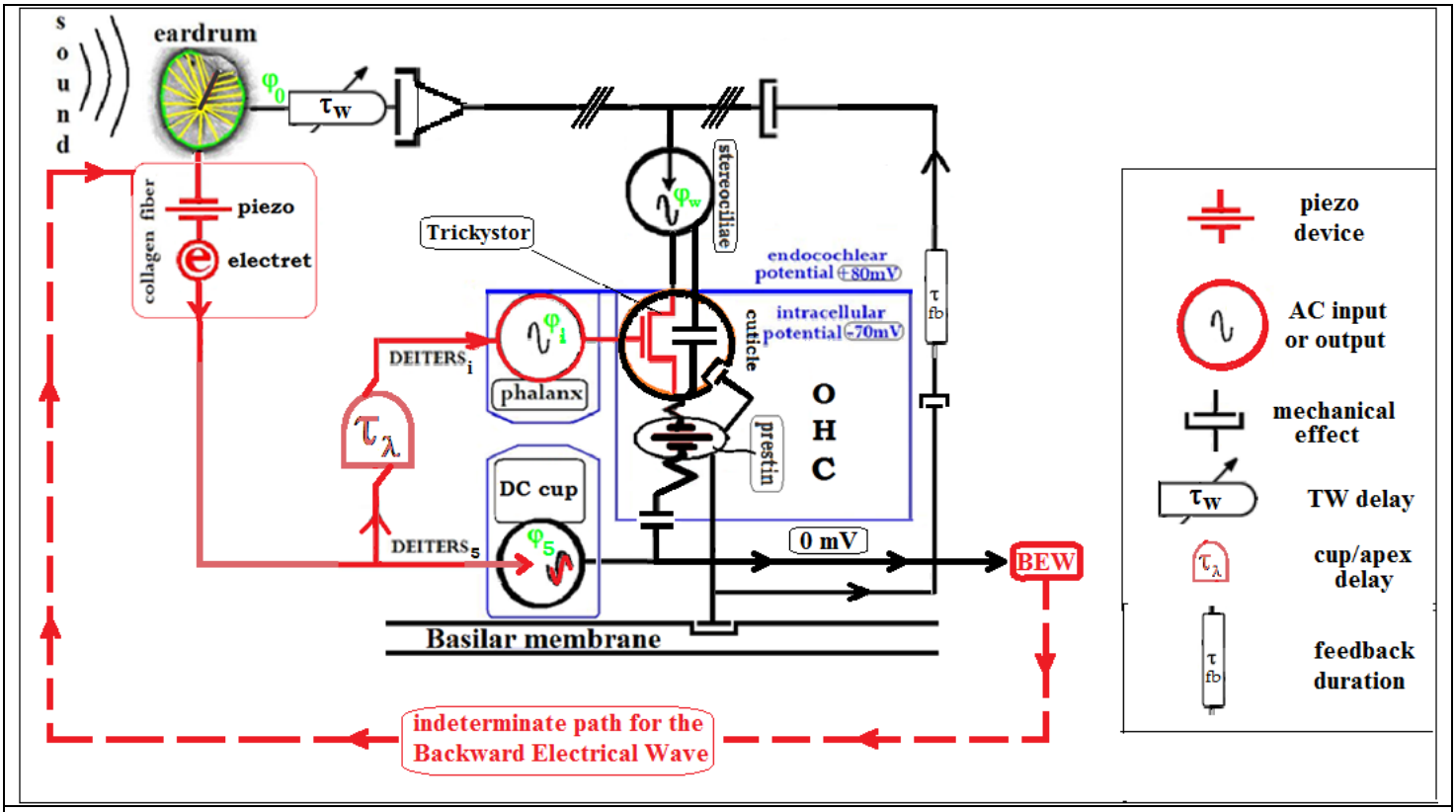


Figure 16

The "covert" pathway is in red; black components refer to both "overt" and "covert" pathways.
τ_{fb} is the delay caused at each loop by the feedback process, thus causing a progressive desynchronization.

The sound signal (from left to right) comes from the outside world; the acoustic vibrations pass through the external auditory conduit and reach the eardrum, setting it in motion. We will first analyze the classical way and, secondly, the contribution of the covert path.

Classical pathway *(or overt path)*

Acquired knowledge

The movements of the eardrum, amplified by the ossicular lever, cause the stapes to vibrate, thus creating a TW in the basilar membrane and liquid of the vestibular ramp.

The frequency (f) of the tympanic acoustical stimulus determines the distance (d) of the maximum of the TW from the oval window: the lower the frequency (f) the more distant the maximum of the TW from the oval window. The TW mobilizes the stereociliae of the OHCs after a delay $\tau_{w(f)}$ that increases with d ([242]). The mobilization of the stereociliae creates an alternating electrical signal (S_{cil}), which is transmitted to the inner aspect of the prestin through a cuticular capacitor. S_{cil} (combined with S_{cup}) mobilizes the prestin, which alternately lengthens and shortens the OHCs.

The force generating mechanism, which mobilizes the prestin, is located in the plasma membrane of the lateral wall, where the transmembrane electric field is converted directly into mechanical force[243].

The "covert path"

We propose that this schema must be completed by the "covert path" :

The acoustic movements of the collagenous fibers of the eardrum engender a piezo-electric synchronic potential at this level. Through the GJs of the petrous bone (not represented) and of supporting cells, this piezo-tympanic signal (pT) reaches the apexes of four DCs in the vicinity of the OHCs relevant to the frequencies in question.

This occurs in two ways.

The piezo tympanic signal reaches a DC_5 cup, which supports the OHC in question. DC_5 is distinct from the four other $DC_{i\ (1..4)}$, the phalanxes of which are in contact with the cuticle of the OHC. The cup of DC_5 transmits the pT with a negligible delay ($\tau_{d5} \approx 0$) to the extracellular slot (Cupular signal or S_{cup}) and, through it, to the external surface (extracellular side) of the prestin of the OHC.
At the same time, this pT signal is carried by the cytoskeleton of the phalanxes of the four $DC_{i(1..4)}$ ([28]) toward the TJs (not represented). Even though there is no electrical conduction between the phalanxes and OHCs, there is a capacitive effect (S_{cut}) between phalanxes and anionic cholesterol rafts within the intra-cuticular membrane space.

The cuticular bilayer comprises a great many PUFAs and we assume that they can behave as semiconductors. This property makes the phalangeo-cuticular structure similar to an FET (*Trickystor).*
A similar functioning could be achieved through ion channels, the opening of which would be controlled by the effect of the electric field of phalangeal origin on the cholesterol rafts as a ligand for an intra membrane 'gate'.

The characteristic frequency of the DOHC complex is inversely related to its distance from the oval window (Greenwood relation), the length of the phalanxes of the DC_is , and the length of the OHC[29].
For the classical pathway, the duration of the mechanical signal path increases with the distance between the eardrum and the Greenwood area corresponding to a given frequency.
For the hidden pathway, the duration of the electrical signal path increases with the length of the phalanx, and depends on characteristics of the cytoskeleton that are still not well understood[244].
If the S_{cil} (ciliary signal) and the S_{cut} are approximately synchronous ($\tau_d \approx \tau_w$), this results in an amplified mechanical signal (S_{amp}).
If there is a precession either of the Scut signal on the Scil signal, or of the Scil signal on the Scut signal, the *trickystor* does not immediately acquire effectiveness since this effectiveness requires synchronous interaction of both signals.
The acoustic signal (TW) is transduced into an electrical signal by the stereociliae; this electrical signal is amplified by the *trickystor*; then it is once more transduced into a new overamplified mechanical signal by prestinic movements. These movements act on the basilar membrane and the supra-reticular region in such a way that the local amplitude and accuracy of the TW are enhanced. This feedback process could set up a phase

[28] Cf Beurg, M., Bouleau, Y. and Dulon, D. (2001), The voltage-sensitive motor protein and the Ca2+-sensitive cytoskeleton in developing rat cochlear outer hair cells. European Journal of Neuroscience, 14: 1947–1952. doi: 10.1046/j.0953-816x.2001.01826.x
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lag[245](τ_{fb}), increasing with each cycle. The increasing phase lag would tend, by progressive desynchronization, to lead to self-limitation, damping and extinction of this type of amplification.

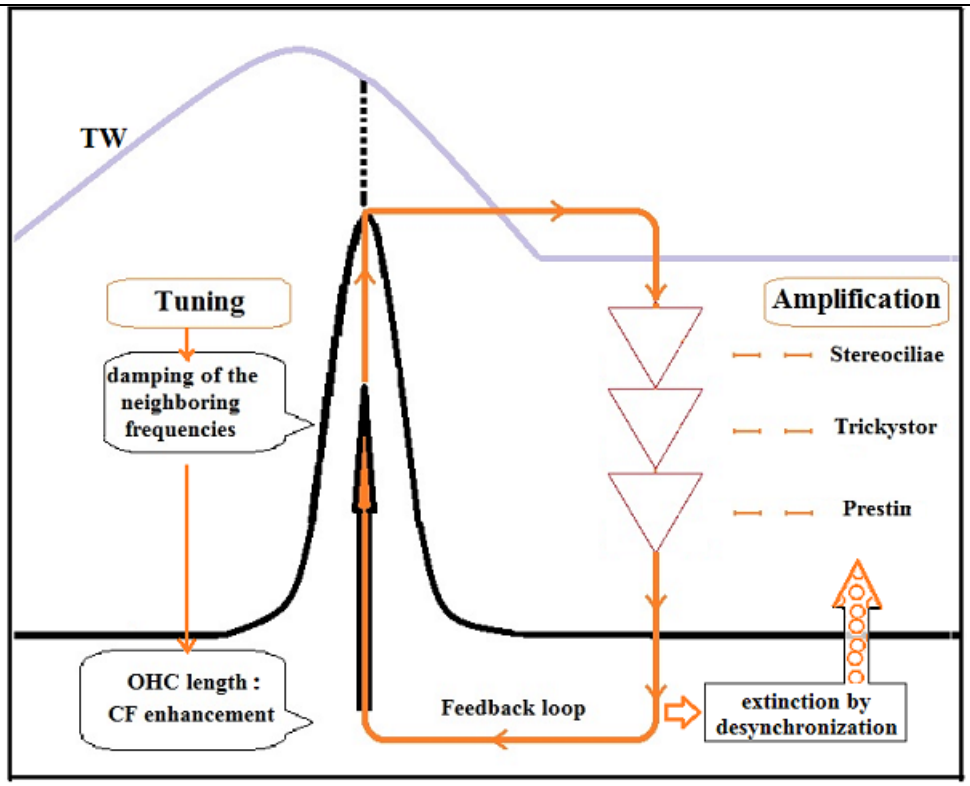

Fig. ???
Schematic view of some putative processes regarding amplification and tuning by DOHC

Conclusion

The tympanum has piezoelectric properties that engender an electrical signal in response to acoustic vibrations ; this signal is, then, carried to the outer wall of the cochlea and from there to the DCs by means of a pathway of an electrical nature. This pathway is made possible by various GJs and connexins. The genetic alteration of these connexins results in non-syndromic deafness.

The piezoelectricity of the tympanum opens up the perspective of an electrical synergistic pathway of sound transmission heretofore unknown (*the covert path*). This pathway is capable of contributing significantly to hearing, especially to hearing the highest frequencies, as it has a determining effect on the amplification and tuning attributed to the OHCs. Our hypothesis of an electric pathway does not negate the established theory of sound transmission but rather expands it, for it is our belief that the mechanical transmission and the electrical transmission of sound work together ([246, 247]) to produce optimal hearing.
The discovery of this electrical transmission of sound will elucidate certain as yet unexplained phenomena of auditory physiology and lay the groundwork for a better understanding of OAEs [elusive backward traveling wave ([248, 249])], amplification and tuning of the cochlear amplifier, presbyacusis, etc.

Thus, our findings have important implications for both theory and practice.
Further experimental studies are needed in order to validate this model and its potential consequences.

Author contributions :

Proposed the idea of the "covert path" (with piezotympanic source), did the physiological theoretical work and conceived the experiments : Bernard M. Auriol.
Designed the experiments : Bernard M. Auriol, Jerome Beard, Jean-Marc Broto, Didier Descouens and Claire Thalamas;
Performed research : Bernard M. Auriol, Jerome Beard, Jean-Marc Broto, Didier Descouens, Christian F. Gillieaux, Bernard Libes;
Analyzed data : Bernard M. Auriol, Jerome Beard, Jean-Marc Broto, Lise Durand, Frederick Garcia;
Wrote the paper : Bernard M. Auriol, Jerome Beard, Lise Durand, Elizabeth Joiner.
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Dans un email, Fabien Picaud m'indique : « *Les flux ioniques déduits sont compatibles avec ce que l'on peut trouver dans la littérature mais on est plutôt dans la moyenne basse* ».
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