Physiological Mini Reviews

6
Volume

Vol. 6 #6, October-November, 2013
ISSN 1669-5402 (Print) | ISSN 1669-5410 (Online)
pmr.safisiol.org.ar

Physiological Mini Reviews

SAFIS
Sociedad Argentina de Fisiología

Physiological Mini-Reviews

[ISSN 1669-5402 (Print); ISSN 1669-5410 (Online)]

Edited by **the Argentinean Physiological Society**

Journal address: Centro de Investigaciones Cardiovasculares y Cátedra de Fisiología y Física Biológica. Facultad de Medicina; Universidad de La Plata; La Plata, Argentina.Tel.-Fax: (54) (0)211 4834833
http: //www.mini.reviews.safisiol.org.ar

Physiological Mini-Reviews is a scientific journal, publishing brief reviews on "hot" topics in Physiology. The scope is quite broad, going from "Molecular Physiology" to "Integrated Physiological Systems". As indicated by our title it is not our intention to publish exhaustive and complete reviews. We ask to the authors concise and updated descriptions of the "state of the art" in a specific topic. Innovative and thought-provoking ideas are welcome.

Preparation and Submission of manuscripts:
"Physiological Mini-Reviews" will have a maximum of 2500 words, 30 references and 4 figures. Material will be addressed to scientific people in general but not restricted to specialist of the field. For citations in the text see Instructions in the electronic page. Final format will be given at the Editorial Office. Most contributions will be invited ones, but spontaneous presentations are welcome. Send your manuscript in Word format (.doc) to: pmr@safisiol.org.ar

---

The “Sociedad Argentina de Fisiología” is a registered non-profit organization in Argentina. (Resol. IGJ 763-04)

HAIR CELLS OF THE MAMMALIAN COCHLEA: EXTRAORDINARY NANOMACHINES

Juan D. Goutman[1] & A. Belén Elgoyhen[1,2,*]

[1]Instituto de Investigaciones en Ingeniería Genética y Biología Molecular, Dr Héctor N Torres (CONICET-UBA).

[2]Tercera Cátedra de Farmacología. Facultad de Medicina. Universidad de Buenos Aires.

***Correspondence to:**
Dr. B. Elgoyhen (elgoyhen@dna.uba.ar)

ABSTRACT

In mammals, the sense of hearing relies on the normal function of two types of specialized cells: inner hair cells (IHC) and outer hair cells (OHC). They both possess the capacity to detect and convert mechanical movements within the cochlea, associated with sounds, into electrical potentials. A set of stereocilias in their apical end is where the mechano-electrical transduction actually occurs. IHC and OHC have very different functions within the complex process of hearing. IHC are responsible for transmitting the electrical information to the brain, for which they possess a specialized glutamatergic synapse with very unique properties. Neurotransmitter is released without pause and with high temporal precision, taking advantage of a synaptic organelle called the ribbon. OHC are exquisite piezoelectric devices, as changes in their membrane potential produce measurable changes in length. This capacity of OHC provides refined frequency selectivity and extra sensitivity to low intensity sounds. Interestingly, OHC function is regulated by an inhibitory innervation that descends from the brainstem and is mediated by acetylcholine. A very special type of nicotinic receptor, $\alpha 9\alpha 10$, participates in this synapse. Recent advances have shed light on the importance of this efferent control on acoustic trauma and higher hearing capabilities.

Keywords: Hair cells, Corti organ, glutamatergic synapsis, acetylcholine, nicotinic receptor.

Original received: December 3, 2013 Revision received December 20, 2013 Accepted: December 27, 2013

Introduction

In everyday life, one is bombarded with acoustic stimuli of different intensities, frequencies and temporal structures. Some may be deleterious for the normal function of the ear, some may be very salient and easily detected, and some may require refined cerebral processing to extract the necessary information about the environment. The neural circuits that compute the acoustic information are located in the brainstem and in higher auditory nuclei up to the auditory cortex [1]. But the sophisticated machinery responsible for the detection of all different sounds and the conversion from mechanical energy intro electrical potentials is located in the inner ear, within a bonny structure called the 'cochlea' (**Figure 1A**) [2].

The cochlea is a complicated labyrinth where fluid movements are produced by acoustic stimulus. The organ of Corti is the epithelium within the cochlea where sensory cells, supporting cells and also synaptic connections to and from the brain, all interact to make hearing happen (**Figure 1B**). The organ of Corti lies over an acellular membrane, called the basilar membrane, with unique mechanical properties. The basilar membrane has the capacity to vibrate in response to fluid movements in the cochlea and propagate this mechanical energy to the organ of Corti. A very stereotyped organization in the cochlea determines that some regions are more sensitive to low frequency sounds and others to high frequency sounds. This spatial segregation mirrors in all cerebral nuclei responsible for processing acoustic information and is called 'tonotopy'.

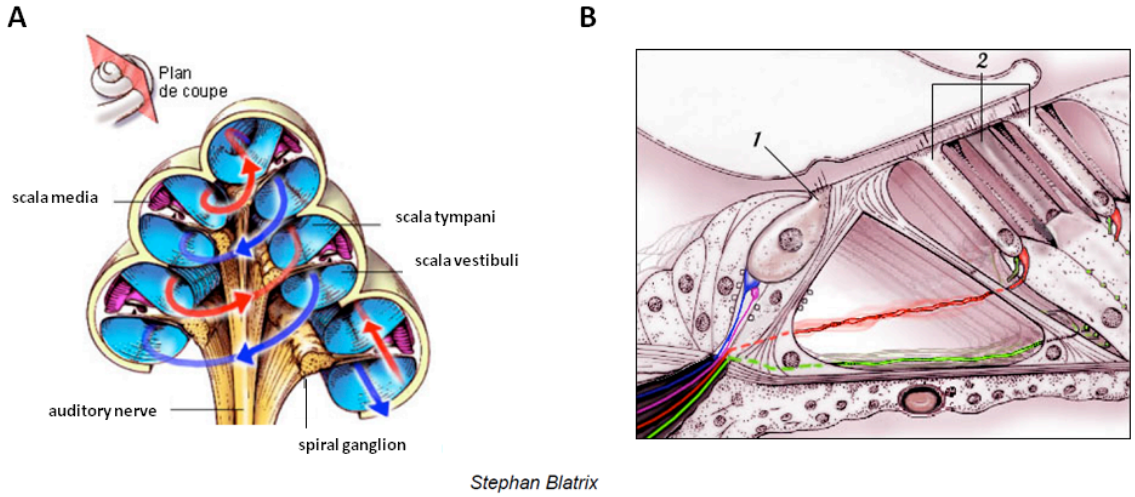

Figure 1: Structure of the cochlea and the organ of Corti. **A,** Cross-section of the cochlea (top inset shows diagram of the whole cochlea and sectioning scheme). The three compartments composing the cochlea are indicated (scala timpani, scala vestibuli and scala media). The organ of Corti is located in the scala media. The spiral ganglion comprises the somas of auditory nerve neurons innervating IHC. **B,** Structure of the organ of Corti. Inner [1] and outer [2] hair cells are indicated. Afferent dendrites belonging to auditory nerve neurons are indicated in blue, and medial olivary complex neurons in red. Some efferent fibers (pink) also make axo-dendritic contacts with afferent boutons. Green fibers represent a small proportion (type II) of afferent contacts on OHC. Image by Stephan Blatrix from "Journey into the World of Hearing", www.cochlea.eu, by Rémy Pujol et al., NeurOreille, Montpellier, France).

Two types of sensory cells in the organ of Corti are unique to the mammalian sensory system: inner hair cells (IHC) and outer hair cells (OHC), indicated in **Figure 1B** [2]. They

are both capable of performing mechano-transduction, but whereas IHC are responsible for relaying acoustic information to the brain, OHC have the fundamental capacity to amplify low intensity stimuli to make them detectable. Both hair cells present a polarized structure, with hair-like stereocilia in their apical end, and synaptic connections on the base. Between 20 and 300 stereocillia exist in each hair cell, the number varying depending on the location within the cochlea, but always in a three row array distribution with different heights [3]. **Figure 2A** shows a scanning electron-micrograph with a top view of IHC and OHC stereocillia. Each stereocillia is formed by a pack of actin filaments and inserts in the apical end of the hair cell within the so called cuticular plate. Stereocillia are deflected by sound waves propagating inside the cochlea, not individually but concertedly in a block, ensuring activation of the mechano-transduction (MET) apparatus. But how does MET actually occur?

Mechano-transduction

The centerpiece in this complex process is an extensively investigated cationic channel with large conductance, and mechano-sensitive characteristics[4]. It has been shown that movements of the stereocillia produce a large inward current in hair cells, with an ultrafast temporal signature[4]. Unfortunately, and even in this post-genomic era, this channel still lacks molecular identification. For many years it has been proposed that a channel of the 'transient receptor potential' (TRP) family could be the pore forming molecule[5]. Some of the TRP channels, such as TRPA present high conductance, high calcium permeability and are mechano-sensitive[5]. However, this hypothesis could not be confirmed and newly presented evidence indicate that members of the *transmembrane channel-like* family would be, at least, part of the channel complex [6].

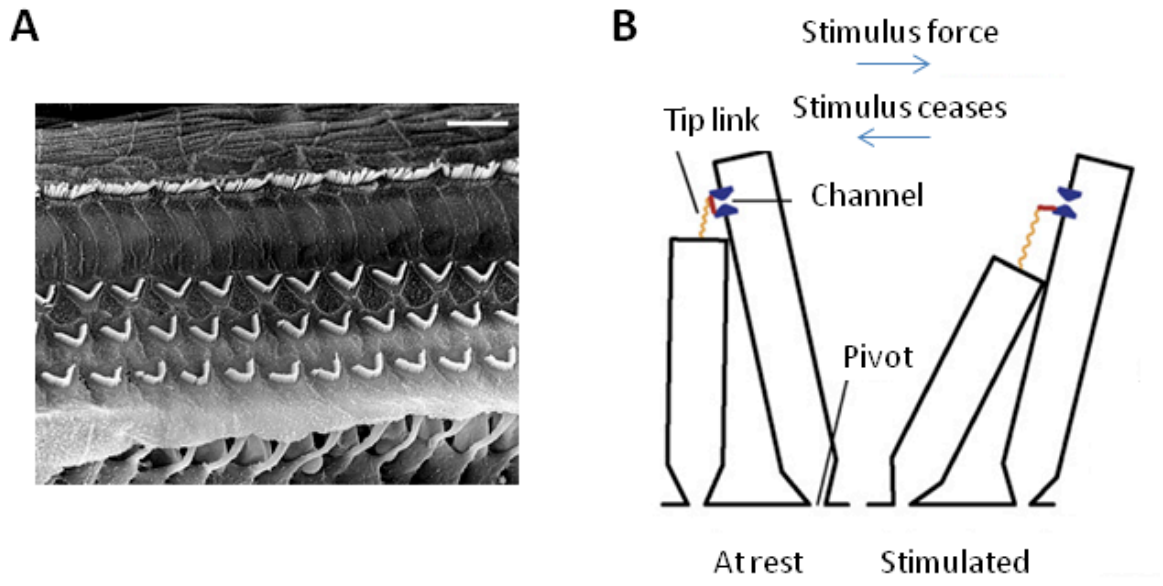

Figure 2: Stereocillia and mechano-transduction: **A,** Scanning electron-micrograph of the surface of the organ of Corti. On the top, the row of IHC stereocillia. The three bottom rows belong to OHC. Scale: 15 μm. Bellow the outer hair cells plane, processes of Deiter's cells can be noted. Image by M. Lenoir from "Journey into the World of Hearing". **B,** Mechano-transdcution scheme. In the left diagram, two stereocillia (for simplicity) are illustrated at resting state, connected by a tip link. The tip link is attached to a transduction channel. Deflection of the stereocillia, produced by mechanical force, pulls open the MET channel and activates the current trough the channel (Modified from [3]).

When mechanical force is applied, either by experimental means or by acoustically evoked fluid movements in the cochlea, stereocillia do not flex but pivot at their base (**Figure 2B**) [3,4]. Once force ceases, stereocillia return to their resting position. This mechanical deflection elicits ionic currents at the hair cell, by gating of the mechano-sensitive channel. One of the main difficulties in the identification of these channels is that there are very few in each cell. Evidence from variance analysis and imaging indicates that one or few channels are located at the tip of each stereocillium, making about 20 per hair cell [7]. The amplitude of the MET current follows a sigmoidal function with respect to the deflection level [3]. Interestingly, the amplitude of the current at the bundle resting point is actually not zero, but approximately 20 - 40 % of the maximum. MET current increases with deflections towards the tallest stereocillia, and decreases in the opposite direction. The conductance of the MET channel also changes along the tonotopical position within the cochlea, suggesting differential requirements at different frequencies.

Several pieces of the MET puzzle have been identified in the past years. Channels are located at the tip of the stereocillia and gating occurs when a spring-like element is stretched due to the stereocillia deflection. Detailed electron micrographs show the presence of connecting threads between each stereocilia and its neighbor from a different row [8]. They are called 'tip links' and calcium buffering agents (such as BAPTA) produce a reversible but complete elimination of this structure [9]. In this situation, MET responses are totally abolished. Genetic screening of patients with Usher syndrome, a devastating sensory disorder, helped determine the molecular identity of the tip links [9]: cadherin-23 and protocadherin-15. They are transmembrane proteins presenting long extracellular domains with several cadherin repeats and are located at the tip links.

In OHC, MET is coupled to the extraordinary capacity of these cells to amplify sound vibration. The mechanism of amplification is a matter of hot debate, although it is clear that OHC can perform 'piezoelectric-like' movements [10, 11]. When they are subjected to a change in membrane potential, OHC are able to elongate (or contract) within milliseconds, and return to their original position at resting potential. The 'piezoelectric actuator' behind this behavior is a transmembrane protein of the chloride transporters family, called prestin [12]. Conformational changes driven by the movements of chloride ions in the interior of this protein would facilitate the elongation or contraction of the cell. Ultimately, these cellular movements would push the basilar membrane further apart in a 'cycle-by-cycle' way, to produce amplification of a low intensity sound wave [11].

Afferent synapse

All aspects of sound, including intensity, pitch, and location of the source are imparted to the brain through the afferent synapse on IHC. In all these situations synaptic vesicles are exocytosed with high temporal precision and without pause [13]. Several synaptic specializations suggest that this synapse evolved in a unique manner to cope with these tasks. One of its main features is a specialized synaptic organelle, called the 'ribbon', that appears as a dense structure attached to the membrane of IHC in electron-micrographs, always surrounded by vesicles (**Figure 3A**) [14]. Other sensory synapses in the retina and vestibular organs present similar characteristics.

Classically, in chemical synapses, action potentials propagate into a synaptic terminal generating calcium entry through calcium channels, which in turn catalyses the fusion of neurotransmitter-filled vesicles [15]. The IHC afferent synapse (also called ribbon synapse) is different. Like in other ribbon synapses, synaptic release occurs in response to graded changes in presynaptic membrane potential, not action potentials [14]. These

changes are driven by the MET current, and therefore produced by acoustic stimuli [16]. IHC present voltage-activated calcium channels of the L-type, which allow calcium in the cell and trigger the release of synaptic vesicles [14]. The characteristics of these channels, low voltage threshold and little inactivation, allow IHC to transmit sensory information in a very precise manner [13]

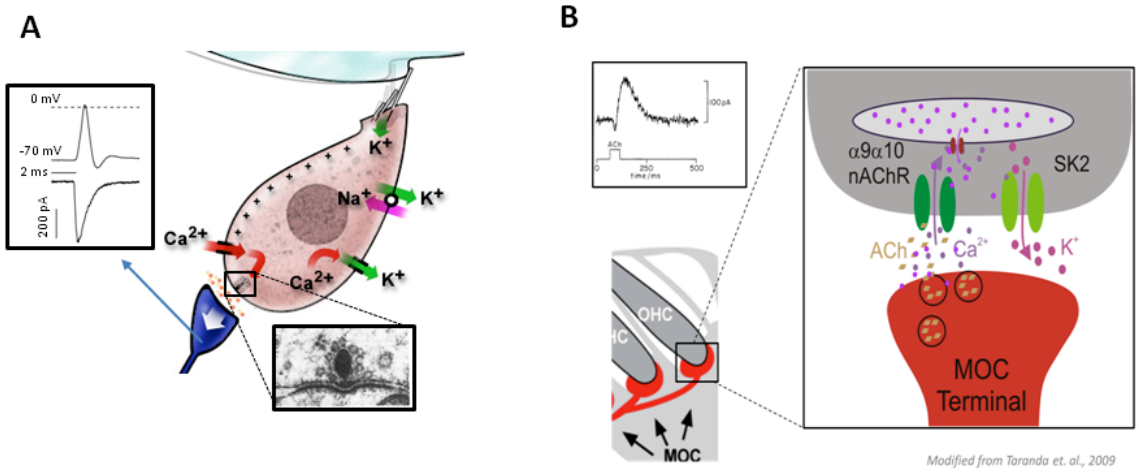

Figure 3: Afferent and efferent synapse on hair cells of the cochlea. **A,** Scheme of the IHC afferent synapse. A postsynaptic bouton of auditory nerve neuron is included in blue (size is overemphasized for illustration purposes). Between 10 to 20 neurons innervate each IHC. A synaptic ribbon is observed opposing to the postsynaptic bouton. An electron-micrograph of the ribbon is shown in detail. Calcium influx, through voltage-activated calcium channels, is coupled to release of synaptic vesicles. Inset: detail of a synaptic current elicited by the release of a glutamate-filled vesicle, and also a synaptic potential followed by an action potential. Modified from "Journey into the World of Hearing". **B,** Detail diagram of the synaptic terminal of MOC neurons onto OHC. MOC terminals release acetylcholine, which activates α9α10 nicotinic receptors in OHCs. Calcium influx through these receptors activates SK channels. A calcium store (included in the diagram) is always observed in direct opposition to the location of the nicotinic receptors. Top inset: typical responses of hair cell to the application acetylcholine. Note the initial and small inward current (carried by the nicotinic receptors) followed the larger outward component due to SK activation. Modified from [39]

Typically, between 10-20 afferent neurons innervate each IHC with individual synaptic contacts, operating independently of each other [13]. These synapses present both overlapping and differential features. All synapses have a basal activity in the absence of sounds, driven by the opening of calcium channels at IHC resting membrane potential (approximately -60 mV) [16]. Upon acoustic stimulation activity increases, signaling to the brain the arrival of a given stimulus [17]. During prolonged stimulation (hundreds of milliseconds) all ribbon synapses at the IHC present depression of release[18]. The intensity of the sound is encoded in the rate at which vesicles are exocytosed, or equivalently, the firing rate of the postsynaptic neurons forming the auditory nerve. But interestingly, the amplitude of the postsynaptic currents is calcium independent[18]. Different neurons contacting a given IHC present different basal activities, different thresholds for sound activation, and they do not saturate at the same intensity [17]. Therefore, it is thought that even if synapses belong to the same IHC, they would operate differently by mechanisms that are not totally established yet.

The IHC afferent synapse is glutamatergic, and is mediated by AMPA type receptors present in the postsynaptic boutons of auditory nerve neurons [13]. No NMDA component has been found in the normal operation of the synapse, although in certain conditions of intense acoustic stimulation NMDA receptors are transiently expressed [19]. As in other

synapses in the auditory pathway, neurons show synaptic adaptations to efficiently respond to high frequency stimuli [20]. For instance, synaptic currents are fast, with decay times of < 0.5 ms, and neurons show low input resistance which ensures fast synaptic potentials, little synaptic integration, and high fidelity responses in a cycle-by-cycle manner (**Figure 3A inset**).

The specialized synaptic organelle present in hair cells, the ribbon, also called synaptic rod or body, is a matter of intense investigation. As indicated, the ribbon appears as an electro-dense body in micrographs, surrounded by clear-core synaptic vesicles. It is thought that by concentrating vesicles in the active zones, the ribbon would increase the supply rate, supporting the capacity of this synapse to continuously release neurotransmitter [14]. The first identified molecular component of the ribbon was only found in this type of structures and was called ribeye [21]. Ribeye is a critical component due to its high predominance in the ribbon. Bassoon, a presynaptic protein found ubiquitously, has a special role at this synapse. It binds to ribeye and is responsible for the attachment of the ribbon to the presynaptic plasma membrane [22, 23]. In bassoon deficient synapses, ribbons appear floating freely in the cytoplasm and synaptic transmission is profoundly impaired [22]. Synaptic currents occur almost normally, but at a lower rate and with a deficient recovery process.

Other molecular components of the release machinery are only partially shared with those of CNS synapses. Synaptotagmin, the canonical calcium sensor present in synaptic vesicles, does not intervene in exocytosis at hair cells [14]. Instead, another candidate called otoferlin, with various calcium binding domains has been identified in a genetic screening from hearing impaired patients [24]. Otoferlin presents high affinity for calcium and also binds to the SNARE complex in a calcium dependent manner, two fundamental properties required for a calcium sensor candidate. Transgenic mice with a null otoferlin mutation show strongly impaired hearing and non functional afferent synapses, albeit structurally normal synapses. Altogether these data indicate that otoferlin has a critical role in synaptic transmission at the afferent synapse, and strongly suggest that it could function as the calcium sensing agent.

It was initially described that neuronal isoforms of the SNARE complex protein suchs as SNAP-25, syntaxin I and synaptobrevin I, were expressed at the IHC afferent synapse [25]. More recently this idea has been challenged by a study indicating that release at this synapse is totally independent of the SNARE complex [26]. More experiments are required to determine how vesicles are exocytosed at the hair cell ribbon synapse.

The afferent neurons that have been described so far contact only IHC, are classified as type I, and represent 95% of all afferents. There is a minor percentage of neurons, called type II, that receive inputs from several OHC with *en passant* synapses [14]. Glutamate also mediates synaptic transmission at these synapses and OHC also present synaptic ribbons, although with different shapes. It is unlikely that type II neurons also encode acoustic information, given that the activity level in these synapses is very low and insufficient to drive neurons to fire in basal conditions [27]. Alternatively, it has been suggested that type II neurons may have a role in coding pain in the ear.

The efferent synapse

Sensory systems of different modalities present connections bringing information about the environment to the brain. The auditory system is unique in that it also presents top-down influences from the CNS, originating in the auditory brainstem, going all the way to the periphery (the cochlea). This efferent innervation controls the sensitivity in the ear, and presents several morphological and functional peculiarities.

Several small nuclei that are responsible for computing auditory information are located in the brainstem and receive inputs originating from both ears. The principal origin of the efferent neurons is the medial olivary complex (MOC) in the auditory brainstem [28].

The main effect of the MOC efferent innervation is to inhibit cochlear responses by decreasing the gain of the cellular amplifier [28]. MOC neurons innervate directly OHC and produce a net hyperpolarization once activated. The neurotransmitter involved in this synapse is acetylcholine (ACh) and the receptor mediating inhibition was a matter of debate over years due to its mixed nicotinic and muscarinic pharmacology [29]. The cloning of two new receptor subunits of the nicotinic family settled the discussion. These new subunits, named $\alpha9$ and $\alpha10$, form ionotropic receptors with the same mixed pharmacological fingerprint: they are not activated by nicotine or muscarine, although both 1-dimethyl-4-phenylpiperazinium (DMPP) and oxotremorine M, nicotinic and muscarinic agonists, respectively, activate the receptor [30, 31]. Receptors formed by $\alpha9$ and $\alpha10$ subunits are cationic and present a high calcium permeability. Once ACh is released from efferent neurons, it opens the postsynaptic receptors in hair cells and it is the large calcium influx that subsequently activates a calcium-dependent small potassium conductance, SK2, producing cellular hyperpolarization (**Figure 3B**, see also inset for detail of the currents) [29]. It is still to be proven if calcium entering through $\alpha9\alpha10$ receptors is sufficient to activate the SK2 channels or if alternatively, calcium coming from the extracellular space triggers the release of more calcium from intracellular stores [32]. Evidence from electron micrographs indicate that this latter possibility is very likely, due to the existence of an endoplasmic organelle in close proximity to the plasma membrane in the basal end of the OHC [29].

MOC fibers respond to sound stimulation increasing their firing rate. From a non-zero basal rate, they can fire up to 100 spikes per second at saturating sound intensity [33]. Inhibition of OHC is strongly dependent on the firing rate of these neurons. It has been shown that short-term plasticity occurs, facilitating the release of ACh and determining a stronger inhibition when MOC neurons are stimulated at higher frequencies [34].

It is important to note that OHC are not the only target of MOC fibers. During postnatal development of the altricial rodent cochlea, IHC transiently receive cholinergic innervation which is also mediated by $\alpha9\alpha10$ receptors [29]. During this period, comprising the first two weeks after birth, mice are deaf, but spontaneous electrical activity in the organ of Corti has been shown to occur. IHC are able to fire calcium action potentials [35], driving neurons in the auditory pathway to fire rhythmically. This activity is determinant for the normal maturation of synapses and circuits of the entire auditory pathway and ceases after the onset of hearing [36]. Activity of the MOC innervation is also inhibitory during this developmental critical period and controls the excitability of IHC [29].

The capacity of the cochlea to detect sounds is greatly deteriorated in the presence of ambient noise, due to increased threshold and reduction of the dynamic range [28]. One of the main functions of the MOC innervation is to reestablish normal parameters in noisy environments. With MOC stimulation, the response to background noise is inhibited, reducing adaptation and restoring IHC capacity to release exhausted synaptic vesicles.

The MOC system also has an important role in acoustic trauma. It is now well established that overexposure to loud sounds causes trauma to the ear, deteriorating our ability to hear [37]. This effect can be temporary (like after attending a rock concert) or permanent (as a result of recurrent loud noise over long periods of time). The consequences of acoustic trauma are diverse but include stereocillia disarrangement, synaptic terminal swellings, and even hair cell death [38]. This latter one is the most critical, given that mammalian hair cells do not regenerate and therefore, loss of cells determines loss of hearing capacity. One important function of the MOC efferent system is the protection to trauma produced by

overly loud sounds. Stimulation of the MOC fibers during sound overexposure produces a reduction of sensitivity loss [37]. A transgenic mouse line carrying a MOC – OHC with increased synaptic strength (through a gain-of-function α9α10 receptor with increased gating properties) presents higher tolerance to trauma [39]. It is still unknown whether protection is due to reduced mechanical vibration of the sensory epithelium, or if calcium entry through α9α10 produces downstream effects impacting on OHC function.

Conclusion

This review provides a succinct overview of different processes occurring in the auditory periphery that are essential to the sense of hearing. Given the peculiarities of this sensory modality, detection of the simplest sound requires a very complex series of cellular events. The biophysical and physiological details of phenomena such as mechano-transduction, non-exhausting afferent synaptic transmission and efferent control of the hearing sensitivity are only starting to emerge.

References

[1] **Brugge JF** (1992) An overview of central auditory processing. In: The Mammalian Auditory Pathway: Neurophysiology (Poppe AN, Fay RR, eds), pp 1–33. New York: *Springer-Verlag* New York, Inc.
[2] **Dallos P** (1996) Overview: cochlear neurobiology. In: The Cochlea1 (Dallos P, Popper AN, Fay RR, eds), pp 1–43. New York: *Springer-Verlag* New York, Inc.
[3] **Hudspeth AJ** (1989) How the ear's works work. *Nature* 341:397–404.
[4] **Fettiplace R, Hackney CM** (2006) The sensory and motor roles of auditory hair cells. 7:19–29.
[5] **Christensen AP, Corey DP** (2007) TRP channels in mechanosensation: direct or indirect activation? *Nat Rev Neurosci* 8:510–521.
[6] **Pan B, Géléoc GS, Asai Y, Horwitz GC, Kurima K, Ishikawa K, Kawashima Y, Griffith AJ, Holt JR** (2013) TMC1 and TMC2 are components of the mechanotransduction channel in hair cells of the mammalian inner ear. *Neuron* 79:504–515.
[7] **Denk W, Holt JR, Shepherd GM, Corey DP** (1995) Calcium imaging of single stereocilia in hair cells: localization of transduction channels at both ends of tip links. *Neuron* 15:1311–1321.
[8] **Kachar B, Parakkal M, Kurc M, Zhao Y, Gillespie PG** (2000) High-resolution structure of hair-cell tip links.
[9] **Richardson GP, Monvel JB De, Petit C** (2011) How the Genetics of Deafness Illuminates Auditory Physiology. *Annu Rev Physiol* 73:311–334.
[10] **Brownell WE, Bader CR, Bertrand D, de Ribaupierre Y** (1985) Evoked mechanical responses of isolated cochlear outer hair cells. *Science* (80-) 227:194–196.
[11] **Ashmore J** (2008) Cochlear outer hair cell motility. *Physiol Rev* 88:173–210.
[12] **Zheng J, Shen W, He DZZ, Long KB, Madison LD, Dallos P** (2000) Prestin is the motor protein of cochlear outer hair cells. 405:149–155.
[13] **Moser T, Neef A, Khimich D** (2006) Mechanisms underlying the temporal precision of sound coding at the inner hair cell ribbon synapse. *J Physiol* 576:55–62.
[14] **Matthews G, Fuchs PA** (2010) The diverse roles of ribbon synapses in sensory neurotransmission. *Nat Rev Neurosci* 12:812–822.

[15] **Kandel ER, Siegelbaum SA, Schwartz JH** (1991) Synaptic Transmission. In: Principles of Neural Science, 3rd ed. (Kandel ER, Siegelbaum SA, Schwartz JH, eds). East Norwalk, CT: Appleton & Lange.
[16] **Russell IJ, Sellick PM** (1978) Intracellular studies of hair cells in the mammalian cochlea. *J Physiol* 284:261–290.
[17] **Kiang NY, Watanabe T, Clark LF** (1965) Discharge patterns of single fibers in the cat's auditory nerve. 35 MIT research monograph N, ed.
[18] **Goutman JD, Glowatzki E** (2007) Time course and calcium dependence of transmitter release at a single ribbon synapse. *Proc Natl Acad Sci U S A* 104:16341–16346.
[19] **Puel JL, Saffiedine S, Gervais d'Aldin C, Eybalin M, Pujol R** (1995) Synaptic regeneration and functional recovery after excitotoxic injury in the guinea pig cochlea. *C R Acad Sci III* 318:67–75.
[20] **Trussell LO** (1999) Synaptic mechanisms for coding timing in auditory neurons. *Annu Rev Physiol* 61:477–496.
[21] **Schmitz F, Königstorfer A, Südhof TC** (2000) RIBEYE, a component of synaptic ribbons: a protein's journey through evolution provides insight into synaptic ribbon function. *Neuron* 28:857–872.
[22] **Khimich D, Nouvian R, Pujol R, Dieck S tom, Egner A, Gundelfinger ED, Moser T** (2005) Hair cell synaptic ribbons are essential for synchronous auditory signalling. *Nature* 434:889–894.
[23] **tom Dieck S, Altrock WD, Kessels MM, Qualmann B, Regus H, Brauner D, Fejtová A, Bracko O, Gundelfinger ED, Brandstätter JH** (2005) Molecular dissection of the photoreceptor ribbon synapse: physical interaction of Bassoon and RIBEYE is essential for the assembly of the ribbon complex. *J Cell Biol* 168:825–836.
[24] **Roux I, Safieddine S, Nouvian R, Grati M, Simmler MC, Bahloul A, Perfettini I, Le Gall M, Rostaing P, Hamard G, Triller A, Avan P, Moser T, Petit C** (2006) Otoferlin, defective in a human deafness form, is essential for exocytosis at the auditory ribbon synapse. *Cell* 127:277–289.
[25] **Safieddine S, Wenthold RJ** (1999) SNARE complex at the ribbon synapses of cochlear hair cells : analysis of synaptic vesicle- and synaptic membrane-associated proteins. *Eur J Neurosci* 11:803–812.
[26] **Nouvian R, Neef J, Bulankina A V, Reisinger E, Pangršič T, Frank T, Sikorra S, Brose N, Binz T, Moser T** (2011) Exocytosis at the hair cell ribbon synapse apparently operates without neuronal SNARE proteins. *Nat Neurosci* 14:411–413.
[27] **Weisz C, Glowatzki E, Fuchs PA** (2009) The postsynaptic function of type II cochlear afferents. *Nature* 461:1126–1129.
[28] **Guinan JJ** (2006) Olivocochlear Efferents : Anatomy , Physiology , Function , and the Measurement of Efferent Effects in Humans. *Ear Hear* 27:589–607.
[29] **Fuchs PA** (1996) Synaptic transmission at vertebrate hair cells. *Curr Opin Neurobiol* 6:514–519.
[30] **Elgoyhen AB, Johnson DS, Boulter J, Vetter DE, Heinemann S** (1994) Alpha 9: an acetylcholine receptor with novel pharmacological properties expressed in rat cochlear hair cells. *Cell* 79:705–715.
[31] **Elgoyhen AB, Vetter DE, Katz E, Rothlin C V, Heinemann SF, Boulter J** (2001) alpha10: a determinant of nicotinic cholinergic receptor function in mammalian vestibular and cochlear mechanosensory hair cells. *Proc Natl Acad Sci U S A* 98:3501–3506.
[32] **Lioudyno M, Hiel H, Kong JH, Katz E, Waldman E, Parameshwaran-Iyer S, Glowatzki E, Fuchs PA** (2004) A "synaptoplasmic cistern" mediates rapid inhibition of cochlear hair cells. *J Neurosci* 24:11160–11164.

[33] **Brown MC, Kujawa SG, Duca ML** (1998) Single olivocochlear neurons in the guinea pig. I. Binaural facilitation of responses to high-level noise. *J Neurophysiol* 79:3077–3087.
[34] **Ballestero J, Zorrilla de San Martin J, Goutman JD, Elgoyhen AB, Fuchs PA, Katz E** (2011) Short-term synaptic plasticity regulates the level of olivocochlear inhibition to auditory hair cells. *J Neurosci* 31:14763–14774.
[35] **Kros CJ, Ruppersberg JP, Rusch A** (1998) Expression of a potassium current in inner hair cells during development of hearing in mice. *Nature* 394:281–284.
[36] **Kandler K, Clause A, Noh J** (2009) Tonotopic reorganization of developing auditory brainstem circuits. *Nat Neurosci* 12:711–717.
[37] **Saunders JC, Dear SP, Schneider ME** (1985) The anatomical consequences of acoustic injury: A review and tutorial. *J Acoust Soc Am* 78:833–860.
[38] **Liberman MC, Kiang NY** (1984) Single-neuron labeling and chronic cochlear pathology. IV. Stereocilia damage and alterations in rate- and phase-level functions. *Hear Res* 16:75–90.
[39] **Taranda J, Maison SF, Ballestero JA, Katz E, Savino J, Vetter DE, Boulter J, Liberman MC, Fuchs PA, Elgoyhen AB, Maison F, Bele A** (2009) A point mutation in the hair cell nicotinic cholinergic receptor prolongs cochlear inhibition and enhances noise protection. *PLoS Biol* 7:e18.

About the authors

Juan D Goutman is Adjunct Investigator at INGEBI (CONICET). His area of interest is synaptic transmission at the inner ear, cellular biophysics and calcium imaging. He got Ph. D. from the University of Buenos Aires and had postdoctoral experiences in USA and Switzerland. He has 12 original papers published on the subjects mentioned above.

A. Belén Elgoyhen is Principal Investigator at INGEBI (CONICET) and Associate Professor in Pharmacology at the University of Buenos Aires. She has published over 60 research papers on structure-function of nicotinic receptors and physiology of hair cells in the cochlea. She has received several awards including: HHMI international scholar, L'Oreal-UNESCO for Women in Science for Lating America, John Simon Guggenheim, Fundación Konex, TWAS in Biology, and 'Investigadora de la Nación Argentina' (Researcher of Argentinean Nation) Currently, she is President of the Argentinean Society for Neuroscience and Associate Editor of the Journal of Association for Research in Otolaryngology.