will repel each other at certain distance scales owing to electrostatic forces. In the presence of multivalent cations, they may instead exhibit adhesion.
Using a flexible glass fiber and photomicrometer to make quantitative force measurements, we investigated the friction and adhesion between individual stereocilia. The charge density of the stereociliary glycocalyx was measured by pairing capillary electrophoresis of individual stereocilia with electron microscopy. Using chemical labeling techniques and fluorophore-conjugated lectins, we identified specific sugars in the glycocalyx. Together, these experiments provide a functional understanding of the hair bundle's glycocalyx and speak to the question of how the hair bundle maintains coherence while simultaneously minimizing friction.

2630-Pos
Coupling a Sensory Hair-Cell Bundle to Cyber Clones Enhances Nonlinear Amplification
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The vertebrate ear benefits from nonlinear amplification of mechanical vibrations by sensory hair cells to operate over a vast range of sound intensities. Hair cells are each endowed with a hair bundle which can oscillate spontaneously and function as a frequency-selective, nonlinear amplifier. Intrinsic fluctuations, however, jostle the response of a single hair bundle to weak stimuli and seriously limit amplification. We report that a hair bundle can effectively reduce noise and enhance amplification by teaming-up with other hair bundles. We implemented a dynamic force-clamp procedure to couple a hair bundle from the bullfrog's saccule to two cyber clones that emulated flanking neighbours. We argue that the auditory amplifier relies on hair-bundle cooperation to overcome intrinsic noise limitations and achieve high sensitivity and frequency selectivity.

2631-Pos
Sound Transduction in the Mammalian Outer Hair Cells: Prestin Activity is Required for Proper Deflection of the Stereocilia Bundle
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The outer hair cell (OHC) body is capable of prestin-driven electromotility leading to force generation that increases the vibration of the hearing organ critical for auditory sensitivity. At the cell's apex, the stereocilia bundle deflects as a unit during sound stimulation (Fridberger et al, 2006). Such a deflection converts nanometric displacements into electrical signals transmitted to the auditory nerve.
Very little is however known about how sound stimuli cause the bundle to deflect, especially, the possible contribution of prestin-induced cell body vibrations to this deflection has never been investigated.
Here we investigated the influence of the membrane protein prestin activity on the bundle deflection, in an intact ear preparation from the Guinea pig. Prestin was previously shown to be specifically inactivated by salicylate and tributyltin. Using an approach combining rapid confocal imaging and optical flow-based computation, the bundle deflection was studied under simultaneous sound stimulus administered at 50-350HZ, a frequency band typical of OHCs vibrations in the apex of the cochlea.
To our surprise and irrespective of the prestin inhibitor used, sound-induced bundle deflection drastically increases, specifically, near the best frequency whose position was altered. Likewise, the vibration of the bundle tip intensified. Moreover, the shape of the bundle deflection's pattern was affected.
Our data challenge the general assumption that prestin inactivation decreases the vibrations of the cochleaŕs structures. Because no consistent change was observed for vibrations of the reticular lamina, the increase in the bundle deflection may be caused by a robust vibration of the top. The data suggest that prestin motor's activity regulates the tuning of the bundle vibrations and may explain how the stereociliary and saumatic amplifiers interact during sound transduction in the mammalian ear.

2632-Pos
Dynamic State and Compressive Nonlinearity of Coupled Hair Cells in the Frog Sacculus
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Auditory and vestibular organs of non-mammals lack outer hair cells yet the organs all exhibit signs of an active process. Active hair bundle mobility has been proposed as the cellular basis for this amplification. Uncoupled hair cells in the bullfrog sacculus exhibit spontaneous mechanical oscillations and a compressive nonlinearity that agrees with theoretical predictions. Using a high-speed CMOS camera we are able to record the motion of many hair bundles in parallel in an in vitro preparation of the bullfrog sacculus. Spontaneous mechanical oscillations are not observed when the hair bundles are coupled to the otolithic membrane implying that the cells are in a quiescent rather than oscillatory regime. We explore the compressive nonlinearity of arrays of cells under native coupling conditions.

2633-Pos
AFM Images of Outer Hair Cells' Lateral Plasma Membrane: An Autocorrelation Function Analysis
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Our atomic force microscopic study of the cytosolic surface of outer hair cells' lateral plasma revealed images of membrane particles with tip-geometry- corrected diameter of ~10 nm [1], consistent with 10-nm particles reported by earlier EM studies. These particles were aligned preferentially in one direction and a much weaker alignment consistent with hexagonal packing. The immunoreactivity of these particles to prestin-antibody revealed that these particles involve prestin, a member of the SLC26 family of anion transporters associated with electromotility of outer hair cells. This observation together with reports that prestin forms tetramers consistent with the dimension of these 10-nm particles prompts a question: Are 10-nm particles tetramers of prestin? To address this question, we examined autocorrelation function of AFM images for the detailed structure of these particles. If the slice plain of the peak is adjusted to the dimension that matches the particles, the contours should reveal shapes of the particle. We found the contour at the corresponding height is approximately square, consistent with tetramer symmetry. However, the maximum width of the central peak corresponded to ~8.2 nm, somewhat smaller than the size of the particles obtained by section analysis. This difference can be attributed to blurring effect of noise. In summary, our observation is consistent with a hypothesis that 10-nm particles are prestin tetramers.
[1] Organization of membrane motor in outer hair cells: an atomic force microscopic study, G. Sinha, F. Sabri, E. Dimitriadis, K. Iwasa, Pfugers Archiv European J. of Physiology, 2009.

2634-Pos
Two Photon Imaging of Calcium Signalling at the Mouse Inner Hair Cell Ribbon Synapse
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The information sent from each cochlear inner hair cell (IHC) to the afferent nerve is determined by 10-20 ribbon synapses, structures specialised for rapid release of vesicles upon cell depolarization. To study the IHC calcium domains during transmitter release in mature wild-type mice, we have imaged and simultaneously measured currents in IHCs through an apical opening in the isolated temporal bone. Cells were recorded on the stage of an upright 2PCLSM at room temperature, superfused with medium containing 2mM Ca^{2+}. IHCs could be visualised either with oblique optics or by using 830nm trans-illumination through bone structures. Using whole-cell tight seal recording, with Cs^{+} containing pipettes to reduce large outward currents, the I-V curve of the IHCs exhibit a Ca current with peak magnitude of approx 80pA near −20mV. To observe the distribution of Ca^{2+} entry in the vicinity of the ribbon sites, cells we pipette-loaded IHCs with either high or low affinity Ca^{2+} dyes (200uM OGB1 or OGB5N respectively) and imaged the basal IHC pole up to maximal rates of 70 frames/s during 20 ms or 100ms depolarizing steps to 0mV. At the fastest rates, the images derived from within single cells showed an initial punctuate rise of Ca^{2+} at the presumed synaptic sites with a larger increase at the neural side, a possible correlate of differing afferent thresholds known to characterise auditory nerve fibres. The sites were correlated with fluorescent hotspot distribution identified by IHC FM-dye uptake. The distribution of sites, the localisation of signal maxima close to (<3um) the plasma-membrane and recovery time constant (~100ms) of Ca^{2+} influx also suggests that intrinsic Ca^{2+} buffering near the ribbon synapse was not significantly perturbed. *Supported by EuroHear, the Physiological Society (SC), and Collège de France (JBM).*

2635-Pos
Exploring the Electrical Resonance's Affect on the Mechanical Oscillations of Hair Cells in the Bullfrog Sacculus
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Under in vitro conditions, uncoupled hair bundles of the bullfrog (Rana catesbeiana) sacculus have been shown to exhibit spontaneous oscillations. We used

a high-speed complementary metal oxide semiconductor camera to track the movements of hundreds of cells in parallel from dozens of preparations. This work revealed that the spontaneous oscillations exhibit multiple timescales with a slow modulation on a rapid oscillation. Experiments inhibiting the electrical resonance in the cell body show a strong effect on the mechanical oscillations of the hair bundles. This indicates that the electrical oscillation is coupled with the mechanical oscillations of the hair bundles.

2636-Pos

Effect of Having Two Identical Channels Per Tip-Link in Hair Bundles

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Gating of mechanoelectrical transducer (MET) channels in hair bundles of hair cells is the key step of mechanosensitivity, including sensing sound and balance. Transducer current, gating compliance, which can lead to negative stiffness of the hair bundle, and adaptation have been successfully described by theoretical models that assume that a single MET channel is connected to each tip-link.

However, recent experimental reports have demonstrated that each tip-link is associated with more than one channel. This discrepancy begs an answer to the question: What are the consequences of two MET channels being associated with a single tip-link?

To address this question, we assume that two channels associated with each tip-link are identical and examine the effect of two basic connectivities, series and parallel, of the channels on their gating. We found that the connectivity has much larger effect on gating compliance than on transducer current. With series connection, gating compliance has a single maximum only if it is below a critical value. Further increase of gating compliance splits its maximum into two. Thus negative stiffness, the result of a further increase in gating compliance, appears in two regions on the displacement axis. In contrast, parallel connection leads to gating compliance similar to that predicted by the previous model that assumes a single channel is associated with each tip-link. Although some cooperative effect of the two channels is present, it cannot be significant for sensitive hair bundles. For those reasons, parallel connection of two identical channels is consistent with experimental observations.

2637-Pos

Structural Determinants of Cadherin-23 Function in Hearing and Deafness

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The tip link is an essential component of the hair-cell transduction apparatus and has been proposed to be part of a biophysically defined "gating spring". Cadherin-23 and protocadherin-15 likely form the tip link; both proteins belong to the cadherin superfamily of proteins that mediate calcium dependent cell-cell adhesion and are involved in hereditary deafness, yet their molecular structures and elasticity are unknown. Here we present crystal structures for cadherin-23 repeats 1 and 2 (EC1+2). Overall, the structures show a typical cadherin fold for both repeats, but reveal an elongated N-terminus that impairs classical cadherin-cadherin interactions and facilitates the formation of an additional calcium binding site. The linker region between the repeats closely resembles a classical cadherin calcium-binding motif and contains the site of the D124G mutation causing non-syndromic deafness (DFNB12). The crystal structure of EC1+2 carrying this mutation displays a different angle between the repeats than the wild-type protein. Molecular dynamics simulations of wild type structures and various mutant models suggest that deafness mutations and removal of calcium ions control cadherin inter-repeat motion and unfolding strength of hair-cell tip links. The structures along with simulations indicate that cadherin repeats forming the tip link are too stiff to be the gating spring. In addition, the new structures define a previously uncharacterized family of cadherin proteins and begin to suggest mechanisms underlying disease as well as ways in which cadherin-23 may bind end-to-end with itself and also with protocadherin-15 to form the tip link.

Ryanodine Receptors II

2638-Pos

Emerging of Ryanodin Receptors Mediated Calium Signaling Synchroonizes Early Cardiac Contractility

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Stem cell transplantation is a promising technique to improve cardiac function. However arrhythmias in regenerating areas are a major concern surrounding the use of cellular cardiomyoplasty. We associate these arrhythmias with developmental changes in intracellular calcium (Ca^{2+}) signaling. Cellular contractility in early cardiac development induced by Ca^{2+} oscillations evoked by Ca^{2+} release from Inositol 1,4,5-triphosphate receptors (IP3Rs), whereas in adult myocytes Ca^{2+} released from the ryanodine receptors (RyR2s) is the main source of cytosolic Ca^{2+} elevation. Recently we have shown that cardiac specific RyR2s mediated Ca^{2+} signaling in early cardiogeneses does exist in early cardiogenesis but functionally silent due to the lack of sufficient luminal Ca^{2+} reserve. Adenovirus mediated calsequestrin (CASQ2) gene transfer in mouse embryonic stem cells potentiated expression of CASQ2 protein in early cardiac development and contributed to the emergence of RyR2s mediated Ca^{2+} signaling in the developing cardiac myocytes. Expression of CASQ2 significantly increased synchronization contractility of early stage (7+1days after in vitro induction of cardiac differentiation from mouse embryonic stem cells cardiac myocytes) in a single cluster of contracted cells (94% of clusters contracted synchronously vs 14% in control). Depletion of RyR2s associated Ca^{2+} stores with caffeine demolished synchronization and reduced frequency of cellular contractility (31 ± 3 vs 48 ± 5 bpm in control). Similar changes were observed in 7+7 differentiation stage cardiomyocytes differentiated from wild type ES cells. Caffeine reduced number of synchronously contracted clusters from 84% to 28%. Frequency of cellular contractility was also reduced from 46 ± 3 to 34 ± 4 bpm.

We conclude that establishment of RyR2s mediated Ca^{2+} signaling in early cardiogenesis is important for synchronization of early cardiac contractility. Ectopic expression of CASQ2 in early cardiogenesis leads to creation of functional RyR2 Ca^{2+} stores and has major impact on stabilization of intercellular contractility.

2639-Pos

Calcium Influx Analysis by TIRF Microscopy on Cultured Primary Myotubes from Patients with *RyR1* Mutations

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Central core disease (CCD) and malignant hyperthermia (MH) have been linked to point mutations in the gene encoding the skeletal muscle sarcoplasmic reticulum calcium release channel (ryanodine receptor), which is localized on human chromosome 19 (*RYR11*). Central core disease is a relatively mild, slowly progressive autosomal dominant myopathy, characterized histologically by the presence of centrally located cores running the length of the muscle fibres. MH is a pharmacogenetic induced hypermetabolic disease. CCD linked RyR1 mutations are associated with depletion of thapsigarin-sensitive stores and to an increase of the resting calcium level. Influx of Ca^{2+} from the extracellular environment is a major factor which influences the level of resting cellular [Ca^{2+}]. Our working hypothesis is that decrease of sarco(endo)plasmic reticulum Ca^{2+} load via leaky ryanodine receptor channels and/or alteration of calcium influx via store operated channels or excitation-coupled Ca^{2+} entry (ECCE), may account for, at least in part, the phenotype of patients with CCD, including muscle weakness and abnormal secretion of inflammatory cytokines from muscle cells and cells of the immune system. We set out to test the validity of our hypothesis by directly investigating the mechanisms activating calcium influx in myotubes from normal individuals and from patients with CCD and MH by TIRF microscopy. Our data shows that mutations in the RyR1 affect ECCE in human myotubes from CCD and MH patients. *This work as supported by grants from A.F.M., S.N.F., Telethon Italy*

2640-Pos

Dysregulation of Ca^{2+} Entry and SR Calcium Leak are Responsible for Elevated Resting Free Ca^{2+} in Triadin-Null Myotubes

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Previously we have shown that lack of triadins expression in skeletal muscle cells results in significant increase of myoplasmic resting free Ca^{2+} ($[Ca^{2+}]_r$), suggesting a role for triadins in modulating global intracellular Ca^{2+} homeostasis. To further characterize this mechanism, here we study the effect of triadin on Ca^{2+} entry, $[Ca^{2+}]_r$, and Ca^{2+} release pathways using Mn^{2+} quench, Ca^{2+} selective microelectrodes and Ca^{2+} imaging in Wt and triadin-null myotubes. Comparison of Mn^{2+} quench rates in resting conditions revealed that triadin-null cells have higher Ca^{2+} entry rates than Wt cells. However, prevention of Ca^{2+} entry with Cd^{2+} and La^{3+} was insufficient to restore