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
Jérémie Barral[1], Kai Dierkes[2], Benjamin Lindner[2], Frank Jülicher[2], Pascal Martin[1].
[1]CNRS - Institut Curie - UPMC, Paris, France, [2]MPIPKS, Dresden, Germany.
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
Pierre Hakizimana, Anders Fridberger.
Karolinska Institutet, Center for Hearing and Communication Research, Stockholm, Sweden.
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
Clark Elliott Strimbu, Damien Ramunno-Johnson, Lea Fredrickson, Dolores Bozovic.
UCLA, Los Angeles, CA, USA.
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
Ghanshyam Sinha, Emilios K. Dimitriadis, Kuni H. Iwasa.
National Institutes of Health, Bethesda, MD, USA.
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
Jonathan F. Ashmore[1], Siân Culley[1], Jacques Boutet de Monvel[2], Christine Petit[2], Said Safieddine[2].
[1]University College, London, United Kingdom, [2]Institut Pasteur, Paris, France.
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
Damien Ramunno-Johnson, C. Elliott Strimbu, Lea Fredrickson, Albert Kao, Dolores Bozovic.
UCLA, Los Angeles, CA, USA.
Under in vitro conditions, uncoupled hair bundles of the bullfrog (Rana catesbeiana) sacculus have been shown to exhibit spontaneous oscillations. We used