

**2625-Pos****Structure Function Studies of the Proton Coupled Folate Transporter**

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Folate vitamins are essential for DNA replication and cellular proliferation. However, mammalian organisms are devoid of folate biosynthesis and rely on dietary sources to meet their metabolic requirement for folate cofactors. The proton coupled folate transporter (PCFT/SLC46A1) has been recently identified as the molecular entity responsible for intestinal folate uptake displaying optimal transport activity at acidic pH. PCFT is also involved in transport of chemotherapeutically used antifolates. Currently, there is limited experimentally derived information about the structure and transmembrane topology of PCFT. Hydrophathy analysis suggests 10 to 12 transmembrane segments for PCFT. The aim of our study is to construct a reliable homology model and study the structure function interplay in more detail experimentally. We have made various DNA constructs with different N- and C-terminal tags for the heterologous expression and purification of PCFT: 1) for bacterial expression of the gene, 2) for expression in oocytes and mammalian cells, 3) for cell free expression using the Membrane Max cell free expression kit. We will screen the expression systems to determine which is optimal for generating suitable quantity / quality protein that will be used for structural studies. We will engineer individual Cysteines to study the topology with the Substituted Cysteine Accessibility Model (SCAM). Accessibility will be assessed by Western Blotting (PEGylation, biotinylation). The lipid-protein interface will be investigated by hydrophobic photoaffinity labeling. Both SCAM and photoaffinity labeling will be used to study substrate pathways. We will complement these studies with functional assays (uptake and two electrode voltage clamping). Results from our structure function studies of PCFT will be used in exploring therapeutic strategies for folate malabsorption and in optimizing antifolate drug therapies.

**2626-Pos****Substrate Specificity of the Aminophospholipid Flippase and Other Phosphatidylserine Binding Proteins**

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The generation and maintenance of the asymmetric distribution of phosphatidylserine across the cell plasma membrane is regulated by an ATP-dependent, substrate specific lipid flippase. Transport activity is strongly dependent on the structure of its preferred substrate, 1,2-sn-glycerophospho-L-serine. With the exception of methylation of the primary amine group, any modification to the structural elements comprising the polar portion of the molecule results in significantly reduced rates of substrate transport. Substrate lipids are stereospecifically recognized by the flippase. The 1,2-sn-glycerol isomer, but not the 2,3-sn-glycerol isomer of PS is transport competent, but the enzyme does not distinguish between the L- and D-serine isomers of PS. This substrate specificity defines the characteristics of the lipid binding site and also provides biochemical criteria for the identification of putative flippases. By searching for ATPases that are stereoselectively activated by PS, a candidate flippase has been purified from human erythrocytes. This enzyme is uniquely activated by PS in both detergent micelles and in phospholipid bilayers, and demonstrates a stereochemical specificity identical to that expressed by the flippase. A member of a new class of P-type ATPases, which have been associated with PS flippase activity, has been expressed in insect cells and purified. This enzyme (ATP8A1) is also activated exclusively by the 1,2-sn-glycerol isomer of PS, regardless of the stereochemistry of the serine headgroup. The substrate specificity expressed by the flippase and these two ATPases is unique among PS-binding proteins, including blood clotting factors, protein kinase C or the macrophage PS receptor, each of which interact only with the L-serine, and not the D-serine isomer of PS. These studies indicate that at least two distinct PS binding motifs have evolved to enable proteins to selectively interact with PS.

**Auditory Systems****2627-Pos****Selective Activation of Vestibular Hair Cells by Infrared Light**Suhurd M. Rajguru<sup>1</sup>, Richard D. Rabbitt<sup>2,3</sup>, Agnella Izzo Matic<sup>1</sup>, Stephen M. Highstein<sup>3</sup>, Claus-Peter Richter<sup>1,4</sup>.

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Optical stimulation using pulsed infrared light is novel method with potential for selectively stimulating a small group of neurons. In present study, we show that infrared light can selectively activate vestibular hair cells and may offer a novel method to investigate biophysical mechanisms underlying the response. In experiments conducted in toadfish, *O. tau*, horizontal semicircular canal afferents showed a mix of inhibitory and excitatory responses evoked by infrared radiation. In a subset of afferents the background discharge rate decreased, while in other afferents the background discharge rate increased with infrared radiation. Excitatory vs. inhibitory afferent responses correlated with the dynamic adaptive properties of afferent responses observed during mechanical stimuli. Primary semicircular canal afferents in the toadfish are known to receive convergent inhibitory (GABA) and excitatory (glutamate) synaptic input from hair cells that ultimately shape the afferent discharge response. The present data indicate that afferents known to synapse on glutamatergic hair cells increase their discharge rate with incident infrared light, consistent with depolarization of hair cells and increased tonic release of glutamate. Acceleration-coding afferents which synapse on combinations of Glutamatergic and GABAergic hair cells were observed to reduce their discharge rate with infrared light, again indicating depolarization of hair cells but, in this case the inhibition was consistent with increased release of GABA. Repeated optical radiation elicited depolarization/repolarization of hair cells. Sensitive afferents were observed to phase-lock their discharge with the pulsed light stimulation. Since the entire epithelium was irradiated, this argues against simple kT thermodynamic modulation of hair cell channel kinetics. The results compel the hypotheses that infrared light stimulation increases the open probability of IR-sensitive ion channels and depolarizes hair cells due to the influx of cations. [Supported by NIH R01 DC06685 (Rabbitt, RD) and NIH grant 1R41DC008515-02 (Richter, CP)].

**2628-Pos****Organ of Corti Micromachine Enables Hair Bundles to Deform the Stiff Basilar Membrane**

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The organ of Corti (OC) is a highly organized structure in the mammalian cochlea that houses the sensory hair cells. It is believed that the OC functions to optimize force transmission from the outer hair cell (OHC) to the basilar membrane and the inner hair cell. Recent studies reveal that the OC cannot be considered as a rigid body and has a complex mode of deformation. We developed a 3-D finite element model of the OC to dissect its mechanics. Geometric and mechanical information was taken from the gerbil cochlea at 2 and 10 mm from the stapes, positions encoding high and low frequencies respectively, and in each case several hundred microns longitudinal extent was simulated. The model included all structurally significant components: OHCs, pillar cells, Deiters cells and reticular lamina. The model was validated by reproducing experimental results on point stiffness and longitudinal space constant measured at the basilar membrane and response to current steps through the OC. Deformation of the OC by two different active OHC forces (the OHC somatic force and the stereociliary-based force) was then simulated. A surprising result was that despite smaller magnitude, the stereociliary-based force (0.1 and 0.7 nN at apex and base) was nearly as effective as the somatic force (10 nN) in displacing the basilar membrane. The results also suggested that for the active forces to work efficiently the radial stiffness of the tectorial membrane must be comparable to or greater than the hair bundle stiffness. Funded by NIH R01 DC01362.

**2629-Pos****Friction and Adhesion in the Hair Bundle's Glycocalyx**

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To enable us to hear high-frequency sounds, the mechanosensitive hair bundles of our inner ears must oscillate and amplify at frequencies up to 20 kHz, overcoming both viscous drag and friction within the hair bundles. How can such movements be accomplished for a lifetime without harming cellular structures and causing hearing loss?

The hair bundle is a cluster of linked, finger-like projections, the stereocilia, emerging from the hair cell's apical surface. Each stereocilium is composed of an actin core surrounded by membrane endowed with a glycocalyx, a layer of glycoproteins and glycolipids. The stereocilia stand apart from one another at their basal insertions, but lean toward one another at their tips, where they are connected along the axis of mechanosensitivity by fragile tip links. For efficient sensory transduction to occur, mechanotransduction channels must open in unison when the bundle is deflected, suggesting that the stereocilia exhibit some form of low-friction sliding adhesion. One possible mechanism by which such sliding adhesion might be implemented is through charged sugars such as sialic acid. If stereocilia are coated with negatively charged sugars, they

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 cochlea's 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, *Pflugers 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<sup>2+</sup>. 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<sup>+</sup> 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<sup>2+</sup> entry in the vicinity of the ribbon sites, cells we pipette-loaded IHCs with either high or low affinity Ca<sup>2+</sup> 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<sup>2+</sup> 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<sup>2+</sup> influx also suggests that intrinsic Ca<sup>2+</sup> buffering near the ribbon synapse was not significantly perturbed. Supported by EuroHear, the Physiological Society (SC), and Coll ge 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