2763-Pos Board B733
Bcl-xl Regulates ATP Synthase Activity at the Inner Mitochondrial Membrane
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Anti-apoptotic BCL-2 family proteins such as BCL-xL play a crucial role in protecting cells from death. High levels of expression of BCL-xL are key to the maintenance of life of certain cancer cells, but whether BCL-xL protects cells from death simply by sequestering pro-apoptotic molecules, or by producing a growth phenotype is controversial. Although adult neurons resist oncogenic transformation, they contain high levels of BCL-xL and overexpression of BCL-xL in cultured neurons causes an increase in the number and size of synapses and an increase in synaptic activity, suggesting that BCL-xL enhances the availability of ATP for synaptic events while also in some way enhancing synaptic growth. We describe herein that in cultured hippocampal neurons, BCL-xL overexpression promotes biosynthetic metabolism, increases mitochondrial growth, and enhances the availability of total cellular ATP by increasing the ATP/ADP ratio. BCL-xL specifically enhances mitochondrial ATP production even while producing a marked decrease in cellular oxygen use. Although BCL-xL is usually thought to function in the mitochondrial outer membrane, our findings suggest that it creates a super-efficient state of cellular energy metabolism by direct protein-protein interaction with the ATP synthase at the inner membrane. We find that recombinant BCL-xL protein increases native brain ATP synthase enzymatic activity and that a pharmacological inhibitor of BCL-xL decreases the enzymatic activity of the synthase complex. In patch clamp recordings of the isolated synthasome ATP enhances membrane conductance and a BCL-xL inhibitor decreases the conductance. Our findings suggest that BCL-xL improves the efficiency of mitochondrial metabolism. This allows the neuron to continue to produce ATP needed for synaptic activity during biosynthesis of synaptic components. It remains to be determined if this type of BCL-xL-induced metabolic phenotype is recapitulated in cancer cells.

2764-Pos Board B734
Large Conductance Potassium Channel In Mitochondria of Endothelial Cell
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It is well established that endothelial dysfunction contributes to ischemia-reperfusion injury of cardiovascular system. This phenomenon can be limited by the ischemic preconditioning. Recently, it was shown that mitochondrial ATP regulated potassium channel activation induced ischemic preconditiong of the endothelium in humans in vivo.
In our study a single channel activity was measured after patch-clamp of the mitoplasts isolated from endothelial cell line (EA.hy926). Mitoplast samples were prepared by addition to a hypotonic solution causing the cristae of the inner membrane to unfold and breaking of the outer membrane. Isotonicity was restored by adding of hypertonic solution. A potassium selective current was recorded with mean conductance 270 ± 10 pS in symmetrical 150 mM KCl solution. Patch-clamp single channel studies showed properties of the large conductance Ca2+−regulated potassium channel (BKCa channel): it was activated by calcium and NS1619 an activator of BKCa channel at micromolar concentration range. These effects were blocked irreversible by iberiotoxin (IbTx), an inhibitor of BKCa channel. Additionally, we showed that inhibitor of mitoKATP channel (ATP/Mg2+ complex) have no effects on observed activity of ion channel.
We conclude that large conductance Ca2+−regulated potassium channels are present in mitochondria isolated from endothelial cell line.
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Auditory Systems

2765-Pos Board B735
Coupled Hair Cells in the Bullfrog Sacculus
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Auditory and vestibular organs have remarkable sensitivities which exceed those of a single cell. We are investigating whether this can be explained by the synchronized response of many coupled hair cells. Using a high-speed (10,000 fps) CMOS camera, we are able to record the simultaneous motion of multiple hair bundles in the bullfrog sacculus in an in vitro preparation with the otolithic membrane left intact. We have measured the amplitude decay, phase locking, and correlations between hair cells under localized mechanical stimuli. The space constant for the amplitude decay has been found to lie in the range of a few hundred microns. Other experiments on this preparation will be discussed.

2766-Pos Board B736
Distribution of Frequencies of Spontaneous Oscillations in Hair Cells of the Bullfrog Sacculus
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Under *in vitro* conditions, free-standing hair bundles of the bullfrog (Rana catesbeiana) sacculus have been known to exhibit spontaneous oscillations. We developed a new method for studying the movements of hair bundles using a high-speed Complementary Metal Oxide Semiconductor (CMOS) camera. The techniques we developed allowed us to probe for correlations between pairs of cells, and to acquire records on over 100 actively oscillating bundles per epithelium. We measured the statistical distribution of the oscillation periods of cells from different areas within the sacculus, and on different epithelia. Spontaneous oscillations exhibited a peak period of 33 ms (+29 ms, -14 ms) and showed a uniform spatial distribution across the sacculus. Latest data will be discussed.

2767-Pos Board B737
Voltage Dependent Interactions of the Outer Hair Cell Motor Protein Prestin
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Outer hair cells (OHCs) possess the unique ability to undergo somatic length change in response to sound evoked alteration of transmembrane potential through a process termed electromotility. The acute sensitivity and frequency selectivity of mammalian hearing is dependent upon this process. Electromotility is driven by the transmembrane motor protein prestin which presumably undergoes a conformational change in response to transmembrane potential changes. Previous work demonstrates that prestin exists in multimeric states in the OHC and when exogenously expressed in mammalian cells. However the role that prestin oligomerization in its voltage-dependent motor function has not been defined. Towards this goal, we explore the role of prestin-prestin interactions by measuring fluorescence resonance energy transfer (FRET) as a function of transmembrane voltage in HEK293 cells co-expressing prestin-CFP and prestin-YFP fusion proteins. Our data show that prestin-prestin FRET efficiency decreases with depolarization over the operating range of voltages relevant to electromotility. Prestin-prestin FRET reaches saturation at depolarized voltages and preliminary data suggest the same at hyperpolarized voltages. Interestingly, when the voltage dependence of the FRET efficiency is modeled by a two state Boltzmann function, the valence of the fit closely agrees with the valence obtained from prestin nonlinear capacitance measurements. Our data suggest that voltage-dependent FRET is dependent on prestin-prestin interactions within or between oligomers. Whether these changes occur from voltage-dependent conformational changes in prestin that alter prestin oligomerization is currently being explored. (This work is supported by an NSF CAREER Award and NIH grant DC008134)

2768-Pos Board B738
The Frequency Range of the Ear Supported by Hair Bundle Motility
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The sensitivity of the ear depends on the mechanical feedback in hair cells. Here we examine two models of hair bundle motility by assuming that the energy gained by the hair bundle motor is greater than the energy loss due to viscous drag in the subtectorial gap.
The channel re-closure (CRC) model (Choe et al., Proc. Natl. Acad. Sci. USA, 1998) leads to a frequency limit $(k_g x_g)^2 \Phi F_m$, where k_g and x_g are gating spring stiffness and gating distance of the mechanoelectric transducer (MET) channel, respectively, Φ the factor that depends on channel kinetics, and $F_m (=Ns^2/(hA))$ the morphological factor with the number N of tip-links per hair cell, the rootlet separation s, the height h of the tallest cilia, and the area A of the gap.
Tinevez-Jülicher-Martin (TJM) model (Tinevez et al., Biophys. J., 2007) leads to the frequency limit of the form $a[bF_m\text{-}1]^{1/2}$ with both a and b depend on transducer stiffness $k_g[1\text{-}k_g x_g^2 P_o(1\text{-}P_o)/(k_B T)]$, which is required to be negative, and friction coefficient of the motor among others.