The limiting frequency estimated for the mammalian ear under realistic conditions is up to 1.2 kHz based on CRC model and is up to 2.8 kHz based on TJM model, lower than the mammalian auditory range. If the mechanical characteristics of the MET channel and the adaptation motor are similar, the limiting frequency could be described by the morphological factor in both models, although TJM model is much more sensitive to the operating point. This feature could be used to explain the auditory range of the avian ears and their tonotopic maps by the morphological factor.

2769-Pos Board B739

Frequency Analysis of Complex Waveforms in Sound Stimuli Discriminated by Human Auditory Cortex

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Recognizing objects in the environment from the sounds they produce is one of the primary functions of the auditory system. Recognition is possible, in part, because acoustic features of sounds often represent physical properties of their sources.

This present study is the first to investigate whether a single omitted frequency component in the onset period produces significant differentiation.

Neural activity of 10 human subjects is recorded coincident with the sound stimulus by whole-head Magneto-encephalography (MEG). In the first set of stimuli constructed from a group of carefully chosen anharmoninc frequency, repetitive ('standard') tones with five selected onset frequencies were randomly embedded by rare ('deviant') tones, all tones having randomly varying inter stimulus intervals. In the deviant tones one of the frequency components was omitted during the onset period relative to the standard tones. The frequency of the test partial of this complex tone was intentionally selected to preclude its reinsertion by generation of harmonics or combination tones due to either the nonlinearity of the ear, the electronic equipment or the brain processing.

In the second set of stimuli, time structured as above, repetitive ('standard') tones with five selected sustained partial frequency components were embedded with rare ('deviant') tones for which one of these selected partials was omitted this time in the sustained tone. As before, the carefully frequency selection again precluded their reinsertion by generation of harmonics or combination tones due to the nonlinearity of the ear, the electronic equipment and brain processing.

By comparing the magnetic field responses (mismatch negativity or MMNm) of the two data sets, the presence of significant mismatch negativity is determined to be due to elimination of a single frequency in the onset period of sound stimuli rather than the sustain period.

Local Calcium Signaling

2770-Pos Board B740

Fluorescence Imaging Of Ryanodine Receptor And Caveolin Distribution In Cardiac Myocytes At 30 nm Resolution

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In cardiac ventricular muscle excitation-contraction coupling arises from Ca^{2+} release via clusters of ryanodine receptors (RyRs) in regions of close apposition between the sarcoplasmic reticulum and surface membranes. The local Ca^{2+} signalling in these regions depends on the nanometer organization of RyRs and other protein clusters. Until recently, however, optical imaging was thought to be limited to a resolution of ~250 nm set by the diffraction of light. We have overcome this limitation using a new technique that allows imaging of conventionally labelled fluorescent samples at much higher resolution. Isolated rat cardiac ventricular myocytes were labelled with specific antibodies against cardiac ryanodine receptors and caveolin-3 and secondary antibodies conjugated to the organic fluorochrome Alexa 488. To visualize the distribution of the labelled proteins at the surface sarcolemma cells were imaged on a total internal reflection fluorescence microscope. We observed that Alexa 488 (and several other organic fluorophores) exhibit reversible photobleaching with recovery times >10s. This observation provided the basis for a new imaging technique we term reversible photobleaching microscopy (RPM). RPM relies on intense illumination to reversibly induce a very long-lived dark state from which single fluorochromes slowly return stochastically. These single molecule fluorescence events were detected with a sensitive camera and each event could be localized with an accuracy of better than 30 nm. From several thousand frames we thus reconstructed ultra-high-resolution images of the distribution of RyRs and caveolin at the surface sarcolemma of the stained cells. Both proteins formed dense clusters with diameters that were generally much smaller than the diffraction limit. Our new approach is applicable to measure the nanoscale distribution of many proteins of interest in excitation-contraction coupling or other signalling processes.

2771-Pos Board B741

Simultaneous Determination of Free Zn(II) and Ca(II) with a Single Fluorescent Indicator

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Fluorescent indicators for metal ions such as Ca(II) and Zn(II) are in large measure responsible for our current understanding of the cell biology of these metal ions and others: the success of this approach has fueled the development of scores of fluorescent indicator systems. The inventors of the pioneering ratiometric fluorescent indicators Fura-2 and Indo-1 (Grynkiewicz, et al., 1985) made clear that they had roughly 100-fold better affinity for zinc than calcium, and more recent results have shown that high zinc affinity is frequently the case with other calcium indicators. By comparison, zinc indicator systems have been described with picomolar zinc affinity that are unaffected by 10 mM Ca, 50 mM Mg. Recent evidence now indicates that some phenomena thought to be calcium-dependent are in fact zinc-dependent (Stork and Li, 2006). To help resolve such questions we sought an indicator that would exhibit unique fluorescence signatures when free or bound to Ca or Zn, such that the free metal ions could be determined simultaneously with a single indicator in the same experiment. We found that Calcium Green 5N exhibits significantly different lifetimes in the three states, permitting free Ca and Zn concentrations to be determined simultaneously.

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2772-Pos Board B742

Applications of Genetically Targeted and Optimized Calcium Sensors

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We have recently developed a series of FRET-based genetically encoded sensors for calcium. These sensors (dubbed "cameleons") are made from cyan and yellow fluorescent proteins combined with engineered calcium sensing elements. These optimized cameleon sensors have now been genetically targeted to specific locations within cells including: the ER, mitochondria, Golgi, nucleus, plasma membrane, and cytosol. In the present work we put these sensors to the test in both standard tissue culture cells and in primary hippocampal neurons. We have now used these sensors to define how ER and mitochondrial calcium handling is altered by mutations in the calcium regulatory protein presenilin. Presenilin is an integral membrane protein that localizes to the ER, secretory pathway, and plasma membrane. There is emerging evidence from a number of research groups that presenilin plays a critical role in modulating ER calcium signaling. Using a combination of genetically targeted and small molecule sensors we have identified how mutations in presenilin alter calcium homeostasis in the ER and calcium release through the IP3R. In another line of research aimed at testing the sensitivity and versatility of our sensors, we have identified localized calcium signals generated upon invasion of a host mammalian cell by bacteria. In this work we will discuss the strengths and weaknesses of genetically encoded calcium sensors and potential avenues for further improvement and optimization.

2773-Pos Board B743

Rational Design of Ca^{2+} Biosensor

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Ca^{2+}, the most ubiquitous signaling molecule in human body, regulates numerous biological functions by fluxing between the subcellular compartments. Quantitative and real-time detection of Ca^{2+} concentration fluctuation in specific cellular environments such as the endoplasmic reticulum (ER) is essential to explore the mechanism of Ca^{2+}-dependent cellular signaling. Currently the reported Ca^{2+} sensors based on natural Ca^{2+} binding proteins are limited due to the perturbation of Ca^{2+} signaling. To overcome these limitations, we report the rational design of Ca^{2+} biosensors by engineering a Ca^{2+} binding site into a single enhanced green fluorescent protein (EGFP). These developed Ca^{2+} sensors exhibit a ratiometic fluorescent signal change after binding to Ca^{2+}, with a Kd value optimal for the measurement of Ca^{2+} in the ER. Metal selectivity of the sensors for Ca^{2+} in comparison with Ln^{3+}, and excessive biological metal ions such as Mg^{2+}, K^{+}, Na^{+} has also been examined. In addition, these developed sensors can be targeted to the ER, and exhibit high potential for living cell imaging. Further, their optical and conformational properties have been investigated using various spectroscopic methods. Moreover,