using biochemical methods. We used fluorescent moment image analysis and spatial intensity distribution analysis (SpIDA) to study the oligomeric state of NBCe1-A in cultured cells expressing the cotransporter and in rat kidney tissue. Both methods allow for quantitative measurement of fluorescent particle densities and oligomerization states within individual images acquired with laser-scanning microscopy. Initially we examined basal membranes of highly adherent CHO K1 cells expressing eGFP-tagged NBCe1-A because of their large surface area. As an independent control of monomeric brightness, we used cells expressing monomeric eGFP anchored to the membrane. Taking into account the recovered values of the monomeric eGFP quantal brightness, we show that NBCe1-A exists in monomeric and dimeric states on the cell membrane. We also used an Alexa488-alpha-bungarotoxin conjugate to label cells expressing an NBCe1A-bungarotoxin binding mutant. As a monomeric control, we immobilized Alexa488 dye on cover slips. The spatial fluorescence intensity fluctuation analysis revealed a similar distribution of aggregates as shown for eGFP data. Moreover, we immunolabeled NBCe1-A in rat kidney tissues as well as in cultured HEK293 cells expressing the cotransporter demonstrating the NBCe1-A is present in monomeric, dimeric and rarely in higher order oligomeric states. These experiments demonstrate for the first time the in situ oligomeric state(s) of NBCe1-A.

2978-Pos

Assessing the Mutagenicity Potential of Multiphoton Excitation during Imaging of Intrinsic Fluorescence from Cells and Tissues

Johanna M. Dela Cruz, Jesse D. McMullen, Rebecca M. Williams, Warren R. Zipfel.
Cornell University, Ithaca, NY, USA.

Multiphoton-excited intrinsic fluorescence emission signals from cells and tissues can be used for metabolic imaging and studies of disease morphology in chronic animal imaging experiments, and has diagnostic potential as a future in vivo clinical imaging tool. However, the signals are generally weak and require high laser intensities for imaging. Consequently, not only are viability studies important, but an investigation into the extent that multiphoton excitation is a mutagenic agent is critical. Information on the magnitude of permissible intensity levels of femtosecond pulsed near-IR light is vital to human clinical applications, yet there are currently no regulations that specifically indicate such parameters. This study is directed towards determining whether multiphoton imaging of cellular autofluorescence using 700 - 800 nm wavelength excitations causes mutations in mammalian cells. The induction of mutation by pulsed laser radiation employed for multiphoton imaging entails a risk of carcinogenicity in living tissue. The assessment of potential laser illumination toxicity was carried out by the hypoxanthine-guanine phosphoribosyl (HPRT) mammalian cell gene mutation assay, which measures mutation at the HPRT gene locus in cells, and is one of a handful of reporter loci that have been used as molecular biomarkers for both human and rodent exposure to mutagens and UV light. Experiments were performed to assess possible mutagenic effects of various intensities of 755 nm, 100 fs laser irradiation. Laser powers ranging from 20 to as high as 100 mW delivered as raster scanned excitation through a 0.7 NA objective for 20 seconds was found to be nonmutagenic to the HPRT gene locus test system, while higher laser powers initiated mutagenic responses.

2979-Pos

Optical Analysis of Calcium Channels at the First Auditory Synapse

Thomas Frank[1], Nikolai M. Chapochnikov[1], Andreas Neef[2], Tobias Moser[1].
[1]Medical school, University of Goettingen, Goettingen, Germany, [2]Max-Planck-Institute for Dynamics and Self-Organization, Goettingen, Germany.

Transmitter release at the first auditory synapse, the ribbon synapse of cochlear inner hair cells (IHCs), is tightly regulated by Ca^{2+}. Using fast confocal Ca^{2+} imaging, we have recently described pronounced differences in presynaptic Ca^{2+} signals between single synapses within the same cell. These Ca^{2+} microdomains differed both in their amplitude and voltage-dependence of activation.

As for the mechanism behind the amplitude heterogeneity, we provided indirect evidence for differences in the Ca^{2+} channel complement, pointing towards a differential regulation of Ca^{2+} channel number (N_{Ca}) across synapses. Moreover, a very simplistic model reveals potential consequences of different Ca^{2+} channel complements for sound encoding at different synapses

In order to directly study synaptic Ca^{2+} channels, we are currently implementing an optical fluctuation analysis approach. Here, we present preliminary results along potential caveats. This work provides a framework of how to further dissect presynaptic Ca^{2+} microdomain heterogeneity - likely being involved in determining the diverse responses of the postsynaptic neurons, which, as a population, encode the huge range of perceived stimulus intensities (sound pressure varying over 6 orders of magnitude).

2980-Pos

Reactive Oxygen Species as Essential Mediators of Cell Adhesion and Migration

Giuseppe Maulucci, Giovambattista Pani, Valentina Labate, Marina Mele, Emiliano Panieri, Massimiliano Papi, Tommaso Galeotti, Giuseppe Arcovito, Marco De Spirito.
Università Cattolica del Sacro Cuore, Roma, Italy.

In recent years reactive oxygen species (ROS) and by extension changes in the intracellular reductive/oxidative (redox) balance have come into focus as major regulators of key cellular functions in both physiological and pathological settings. Traditionally viewed as mediators of cell damage by exogenous noxae, oxygen intermediates have been also recognized of signaling roles downstream of cytokine and mitogen receptors, activated oncogenes, nutrient sensors and pro-apoptotic stimuli, when endogenously generated by a number of intracellular biochemical sources. The signaling properties of ROS are largely due to the reversible oxidation of redox-sensitive target proteins, and especially of protein tyrosine phosphatases, whose activity is dependent on the redox state of a low pKa active site cysteine. Cell spreading, adhesion and migration requires ROS generation and interaction with protein tyrosine phosphatases downstream of adhesion molecules. We have taken advantage of a redox-sensitive mutant of the Yellow Fluorescent protein (rxYFP), employed ratiometrically, to draw real-time redox maps of adhering and migrating cells. A quantitative analysis of redox maps allows to disclose a peculiar spatial organization of the redox environment, providing evidence that intracellular ROS are generated after integrin engagement and that these oxidant intermediates are necessary for integrin signaling during cell spreading, adhesion and migration. Taken together these observation support the application of rxYFP in the subcellular mapping of physiological dynamic redox phenomena involved in signal transduction.

2981-Pos

Real Time Imaging of Endogenous MRNAs during Stress

Junwei Zhang[1], Kohki Okabe[1], Tokio Tani[2], Takashi Funatsu[1].
[1]University of Tokyo, Tokyo, Japan, [2]Kumamoto University, Kumamoto, Japan.

During stress, cytoplasmic mRNAs aggregate to RNA granules such as stress granule (SG) and processing body (PB), where they are sorted and remodeled for reinitiation of translation, degradation or storage. Till now, stress-induced assembly of these granules is mainly studied indirectly by using protein markers, and the real time behavior of endogenous mRNAs in living cells has not been detected directly yet and remained uncertain.

Here we used a linear antisense probe to visualize endogenous cytoplasmic mRNAs in living mammalian cells in order to investigate the dynamics of mRNAs under stress. A Cy3 and biotin labeled poly$(U)_{22}$ 2'-*O*-methyl RNA probe was prepared for the detection of poly$(A)^+$ mRNAs. The probe combined with streptavidin was microinjected into the cytoplasm of COS-7 cells, followed by the inducement of 0.5 mM arsenite stress. We also transfected TIA-1-GFP plasmid into the cells by FuGENE 6 to determine SG. As a result, mRNAs visualized by the antisense probe aggregated to granules during stress and the granules colocalized with SG marked by TIA-1-GFP. Next, the number and size of the granules were studied by real time imaging. mRNAs rapidly aggregated to form clusters within 20 min in response to stress. A large amount of small granules first emerged, gradually gathered to bigger ones about 30 min after the inducement of stress.

In this study, the aggregation of endogenous mRNAs to SG was successfully visualized by using the linear antisense probe. The behavior of endogenous mRNAs in SG will be revealed.

2982-Pos

Imaging Dopamine and Serotonin in Live Neurons with Multi-Photon Excited Ultraviolet Auto-Fluorescence

Arkarup Bandyopadhyay[1], Suman Nag[1], Manoj Kumar[2], Shyamala Mani[2], **Sudipta Maiti**[1].
[1]Tata Institute of Fundamental Research, Mumbai, India, [2]National Brain Research Center, Manesar, India.

Monoamine neurotransmitters are implicated in mood, aggression, reward, and addiction pathways in the mammalian brain. However, visualizing them in live neurons with sub-micron resolution has remained a challenge. It is difficult to label them fluorescently, and their intrinsic ultraviolet fluorescence is difficult to access. Unlike serotonin which can be imaged with three-photon microscopy, dopamine presents a special challenge due to its shorter wavelength (~300nm) emission. We now show that dopamine can be imaged with sub-micron resolution in live brain slices with a combination of a non-epifluorescent collection design, special optical elements, and two-photon excitation with a visible femtosecond laser. Substantia Nigra (SN) tissue sections from the