rat show a group of cells much brighter than those observed in non-Nigral control regions. They contain hundreds of dopamine vesicles/vesicle clusters in the soma, akin to the serotonergic neurons from the Raphe imaged earlier. Drugs such as amphetamine are thought to cause non-exocytotic release of dopamine from the dopaminergic neurons but measuring such dynamics has been difficult. Our time-lapse imaging shows that 100 μM para-chloroamphetamine depletes the total dopamine content of SN neurons by about 25% over a time scale of fifteen minutes without substantial translocation of the vesicles. Imaging neurotransmitters in live cells also allows us to monitor the differentiation of stem-cells into serotonergic neurons over a period of many weeks. Interestingly, we observe that the ability to exocytose serotonergic vesicles arises several days after serotonin starts expressing in these cells. Our imaging techniques therefore provide a visual assay for a more functionally relevant maturation point for the differentiation of these cells, a fact with possible clinical significance for the treatment of various neurodegenerative and injury-related disorders.

2983-Pos

Late Endosomal Degradation of Low-Density Lipoprotein Probed with Multi-Color Single Particle Tracking Fluorescence Microscopy

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The vesicle-mediated degradation of low-density lipoprotein (LDL) is an essential cellular function due to its role in cellular membrane biosynthesis. Using multi-color single particle tracking fluorescence microscopy, we have probed the intracellular degradation of low-density lipoprotein in living cells. The unique aspect of our experiments is the direct observation of LDL degradation using an LDL-based probe that increases fluorescence intensity upon degradation. Specifically, individual LDL particles are labeled with multiple fluorophores resulting in a quenched fluorescent signal. Control experiments demonstrate that enzymatic degradation of the LDL particle results in an increase in fluorescence. The ability to directly observe LDL degradation allows us to determine which vesicle is responsible for degradation and quantify the vesicle dynamics involved in LDL degradation. Visualization of early endosomes, late endosomes and lysosomes is accomplished by fluorescently labeling vesicles with variants of GFP. Transient colocalization of LDL with specific vesicles and the intensity of the LDL particle are measured simultaneously. The measured colocalization durations are then correlated with changes in fluorescence intensity due to LDL degradation. We observe that degradation of LDL occurs in the late endosome. While there are a broad distribution of colocalization durations of LDL with Rab7, a late endosomal protein, only relatively long (>420 s) colocalization leads to the degradation of LDL. These studies, which are the first to directly observe the degradation of LDL within a cell, support a model in which late endosomes are the site of degradation with lysosomes serving as enzyme storage vesicles.

2984-Pos

Hotspots of GPI-Anchored Proteins and Integrin Nanoclusters Function as Nucleation Sites for Cell Adhesion

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Lymphocyte function-associated antigen-1 (LFA-1) is a leukocyte specific integrin that mediates migration across the endothelium and the formation of the immunological synapse. Association of LFA-1 into specific cholesterol enriched microdomains, called lipid rafts, is thought to regulate its activity. These 20 to 200 nm lipid rafts are abundantly present on the cell membrane. However, these length-scales are not available for conventional light microscopy, requiring higher resolution. Near-field scanning optical microscopy (NSOM) uses a sub-wavelength aperture probe to locally excite fluorophores thus providing optical resolution at the nm-scale [1]. Here, we have used single-molecule sensitive NSOM to capture the spatiofunctional relationship between LFA-1 and raft components (GPI-APs) on immune cells. Direct dual-color nanoscale imaging revealed the existence of a GPI-AP subpopulation organized in nanodomains that further concentrated in regions smaller than 250nm, suggesting a hierarchical pre-arrangement of GPI-APs. In addition, integrin nanoclusters reside proximal to these GPI-AP nanodomains, forming cholesterol sensitive hotspots on the cell surface [2,3]. These hotspots function as essential intermediates in nascent cell adhesion, driving the formation of large-scale macrodomains that facilitate firm adhesion [3,4]. Altogether, this well-defined pre-assemblage of proteins might constitute a prominent mechanism exploited by the cell to rapidly and efficiently aggregate distinct nanodomains into larger functional cell surface assemblies.

[1] van Zanten et al. BBA-Biomembranes (2009)
[2] Cambi et al. Mol. Biol. Cell (2006)
[3] van Zanten et al. PNAS (accepted)
[4] Diez-Ahedo et al. SMALL (2009)

2985-Pos

Imaging α-Cell Calcium Dynamics

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Glucagon is released into the bloodstream when glucose reaches threateningly low levels. Its secretion from α-cells, within pancreatic islets of Langerhans, stimulates hepatic glucose release and, therefore, restores proper glycemia. Once normoglycemia is reestablished, glucagon release is inhibited. Impairment of this inhibition has been observed in diabetes mellitus where greater amount of glucagon worsens the chronic hyperglycemic state. However, the mechanisms mediating this glucose suppression of glucagon secretion are poorly understood. Two models have been proposed: direct inhibition by glucose, or paracrine inhibition from non α-cells within pancreatic islets. We report here the use of transgenic mouse lines that specifically express Red Fluorescent Protein within α-cells. This strategy makes it possible to easily identify α-cells and study their intracellular calcium ($[Ca^{2+}]_i$) dynamics by Fluo4 imaging. Our glucagon measurements from flow-sorted α-cells indicate that glucose does not directly inhibit α-cells (+57% increase in glucagon secretion from 1 to 20mM, $p<0.05$, ANOVA). This observation supports the paracrine inhibition model. Influx of calcium ions is a trigger for exocytosis of neuroendocrine vesicles. Since glucagon release from islets is inhibited by increasing levels of glucose, one would naively expect that α-cell $[Ca^{2+}]_i$ would decrease. Imaging of calcium dynamics by fluorescence microscopy demonstrates that glucose mediates an increase in α-cell $[Ca^{2+}]_i$ in intact islets (24.7 ± 3.4% increase in fluorescence intensity from 1 to 20mM glucose, compared to +51.5 ± 5.0% for insulin-secreting β-cells). Meanwhile the percentage of α-cells exhibiting calcium oscillations is comparable at low and high glucose levels (44% of α-cells oscillate during a 5-minute observation period). Furthermore, the shape, frequency, and amplitude distribution of these $[Ca2+]_i$ signals were not modified either. Taken together, these results suggest that suppression of glucagon secretion occurs downstream from α-cell calcium influx, likely at the level of vesicle trafficking or exocytotic machinery.

2986-Pos

DNA Stabilizes Fluorescent Few-Atom Silver Clusters with Unique Photophysical Properties

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Recent studies of DNA-stabilized few-atom Ag clusters (Ag:DNA) indicate that these fluorophores may be well suited for high-resolution imaging techniques requiring optically modulated fluorescence, as well as fluorescence applications using two photon excitation. Ag:DNA exhibit sequence-dependent fluorescence from the blue to the near-infrared, thus DNA sequence presents an enormous parameter space for creating new emitters with optimized properties for fluorescence imaging. Even a very small search through sequence space reveals that single-base mutations in oligonucleotides can change the fluorescence intensity of Ag:DNA solutions by 3000%. We address some basic features of Ag:DNA that will help guide future large-scale searches through sequences space. By correlating fluorescence and mass spectroscopy, we identify emitters comprised of around 10 Ag atoms. We also observe absorbance by the DNA bases as an excitation pathway common to all Ag:DNA emitters. Finally, we discuss our efforts towards producing pure solutions of Ag:DNA at high enough concentrations for structural characterization by NMR.

2987-Pos

Multiphoton Microscopy of Entire Intact Mouse Organs

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Three-dimensional datasets from tissue biopsies may provide critical morphological information that is not readily obtained from traditional approaches to histology using thin physical sections of tissue. Multiphoton microscopy (MPM) provides optical sectioning with penetration into highly scattering materials, ready excitation of intrinsic tissue fluorescence, and access to nonlinear signals such as second harmonic generation (SHG). However, the penetration depth of MPM is typical limited to ~200 microns in many tissues. We present MPM of entire intact, fixed and optically cleared mouse organs. Clearing of tissue is typically incomplete for large tissue samples, however, MPM has sufficient tolerance to scattering to image entire mouse organs. Using macro lenses