functions of Syt1. However, in a number of assays Syt1 and C2AB behave differently, indicating that C2AB may not fully mimic the activity of the full-length protein. Detailed conformational studies of full-length Syt1 have not been reported and in the present work we employ EPR spectroscopy to investigate the state of the linker that attaches the C2A and C2B domains to the vesicle membrane in the full-length protein. CW-EPR spectra and double electron-electron resonance (DEER) distance measurements of single spin-labeled Syt1 indicate that the juxta-membrane linker remains closely associated with the membrane interface and acts to oligomerize full-length Syt1 in the absence of calcium. EPR data also demonstrate that a membrane associated glycine zipper/GXXXG motif in juxta-membrane linker is playing a crucial role in this intermolecular association. Using a total internal reflection fluorescence (TIRF) assay we measure the ability of Syt1 to capture liposomes that mimic the target plasma membrane. The TIRF binding assay shows that the ability of Syt1 to oligomerize through this linker plays a role in the ability of syt1 to interact with target membranes. The membrane binding activity of Syt1 likely plays a key role in triggering membrane fusion. Our detailed structural information provides a basis for understanding the different Ca^{2+}-dependent activities of the full-length Syt1 and the soluble C2AB construct in in-vitro fusion assays that involve isolated reconstituted components of the fusion system.

959-Pos Board B714

Measuring the Impact of Lipid Interactions on the Mobility and Localization of Synaptic Proteins in Live Synapses

Jeremy Dittman, Rachel Wragg, David Snead, Yongming Dong, Jihong Bai, David Eliezer.
Biochemistry, Weill Cornell Medical College, New York, NY, USA.

The presynaptic protein complexin can both promote and inhibit fusion through interactions between its central helix and the SNARE complex. A poorly conserved C-terminal domain (CTD) is also required for inhibition of spontaneous fusion. We found that the CTD binds lipids through a novel protein motif and directs complexin onto synaptic vesicles where it can efficiently engage the SNAREs and inhibit spontaneous fusion. Using in vivo dynamic imaging approaches in C. elegans, we observed that complexin is sequestered within presynaptic terminals through its CTD while its escape rate out of the synapse depends sensitively on synaptic activity. Complexin exhibits reduced mobility in synaptic boutons compared to neighboring axonal regions and its mobility is enhanced when synaptic vesicles were removed, consistent with their role in capture and retention of complexin. Finally, several common lipid-interacting protein motifs were imaged at synapses in the presence and absence of synaptic activity, and the impact of disrupting these lipid-binding domains was quantified. Simple one-dimensional reaction diffusion models were used to quantify the dynamics of protein exchange between en passant synapses.

960-Pos Board B715

Guided Growth of Neurons on Micro-Structured Surfaces

Julia Trahe[1], Jana Hüve[1], Philipp Selenschik[1], Nataliya Glyvuk[1], Anne Gauthier-Kemper[1], Jacob Piehler[2], Jürgen Klingauf[1].
[1]Institute of Medical Physics and Biophysics, Münster, Germany, [2]Department of Biology, Osnabrück, Germany.

Synaptic cell adhesion molecules (SCAMs) are well known to interact across the synaptic cleft of central mammalian synapses. However, their functional role in transsynaptic modulation of the synaptic vesicle cycle (i.e. from the postsynaptic to the presynaptic neuron) is poorly understood. Several families of SCAMs have been characterized at the molecular level. Transsynaptic interactions mediated by SCAMs potentially control initial synapse formation, regulate structural maturation of synapses, modulate basal synaptic function including vesicle endocytosis, and participate in different forms of long-term synaptic plasticity.

In order to better separate pre- and postsynaptic effects we grew neurons on microstructured surfaces, functionalized with SCAM proteins. Glass coverslips were patterned with the Neurexin-binding Neuroligin fragment via microcontactprinting by either coating them with silanes to which we covalently coupled Neuroligin with cysteine tag, or with Poly-l-lysine-polyethylenglycol-HaloTag-ligand, covalently linked to Neuroligin via a HaloTag.

Both approaches lead to controlled and guided growth of neuronal outgrowths. Formation of presynaptic sites was triggered within one to two days. These sites showed a positive staining with antibodies against the active zone markers RIM1/2 and the synaptic vesicle protein Synaptophysin1. They often opposed the structured Neuroligin patches, as revealed by 4Pi microscopy. Such varicosities contained vesicles that could be stained with FM 1-43 upon electrical stimulation. Release of FM 1-43 by repeated stimulation could be monitored by TIRF microscopy, displaying similar kinetics as control synapses.

Formation of synapses on structured surfaces opens up the possibility to study presynapse formation and dynamics under controlled conditions.

961-Pos Board B716

Cerebellar Interneurons use Dendritic Voltage and Calcium Signals to Differentially Extract Information from Synaptic Activity

Alexandra Tran-Van-Minh[1], Therese Abrahamsson[1], Laurence Cathala[2], David DiGregorio[1].
[1]Institut Pasteur, Paris, France, [2]Université Pierre et Marie Curie, Paris, France.

Non-linear summation of synaptic inputs within a dendritic branch endows neurons with multiple computational subunits, favoring different types of pattern detection. To date, most neuronal types have been described to display a supralinear summation of synaptic inputs, due to the activation of NMDAR or voltage-gated calcium channels, with a concomitant supralinearity of local Ca^{2+} signaling. Recently, evidence showed that in dendrites of cerebellar stellate cells, synaptic summation is sublinear, likely resulting from a reduced driving-force for synaptic currents caused by large local depolarizations. We expect these large synaptic depolarizations to cause a smaller fractional change in the driving force for Ca^{2+}, therefore a more linear summation of dendritic Ca^{2+}.

In order to characterize the local Ca^{2+} and voltage responses to synaptic stimulation in cerebellar stellate cells, we combined two-photon targeted stimulation of parallel fibers, glutamate uncaging, and fast two-photon imaging of dendritic Ca^{2+} and voltage. Using fast line-scan imaging and the two-component voltage sensor DiO/DPA , we observed that, in accordance with numerical simulations, the local depolarization in the dendrites of stellate cells in response to the activation of a few synapses is rapid (<2ms), of large amplitude (up to 50mV) and distance-dependent, and is widely spread in the dendrite (several tens of µms). The measurement of Ca^{2+} transients in stellate cells dendrites showed that, in contrast, Ca^{2+} transients are more localized (<10µm), summed linearly in response to paired stimulation of parallel fibers, and supra-linearly in response to synaptic activation by high-frequency trains.

Therefore Ca^{2+} and voltage in dendrites can obey different computational rules, the sublinear summation of voltage contributing to sparse-input detection and shaping the activation of Purkinje cells by parallel fibers, while supralinear Ca^{2+} is likely to contribute to regulation of synaptic plasticity.

962-Pos Board B717

Defects in Synapse Structure and Function in a Fly Model of FUS-Related ALS

Mohammad Shahidullah, Hong Fei, Sylvain LeMarchand, Matthew Dalva, Piera Pasinelli, Irwin B. Levitan.
Thomas Jefferson University, Philadelphia, PA, USA.

Amyotrophic lateral sclerosis (ALS) is an adult-onset neurodegenerative disease that leads invariably to fatal paralysis. Although most cases of ALS are sporadic, about 10% are familial. One gene associated with familial ALS encodes the DNA/RNA binding protein Fused in Sarcoma (FUS). There exists a Drosophila model of ALS, in which human FUS with ALS-causing mutations is expressed in motor neurons. These flies exhibit motor neuron degeneration, larval locomotor defects and early death. Similar phenotypes are observed in flies null for the gene Cabeza (Caz), the fly homolog of FUS. We have examined evoked and spontaneous synaptic transmission at the larval neuromuscular junction, larval motor neuron cell body excitability, and presynaptic active zone structure in these fly models of ALS. The amplitude of evoked synaptic currents is decreased by more than 80% in larvae in which human mutant FUS (R521C) is expressed in motor neurons. A similar decrease in evoked synaptic transmission is seen in Caz1 null flies. Furthermore, the frequency of spontaneous miniature synaptic currents is decreased dramatically in FUS-R521C expressing flies. In marked contrast, recordings from motor neuron cell bodies demonstrate that both wild type and mutant FUS expressing neurons can fire normal action potentials, and the voltage-dependent inward and outward currents in the cell bodies are indistinguishable in wild type and mutant FUS motor neurons. Although confocal microscopic analysis of the larval neuromuscular junction does not reveal gross abnormalities, examination of synapses using super-resolution STED microscopy suggests that presynaptic active zones are aberrantly organized in larvae in which FUS-R521C is expressed in the motor neurons. The results are consistent with the idea that

defects in synaptic structure and function precede, and may contribute to, the later motor neuron degeneration that is characteristic of ALS.

963-Pos Board B718

Upregulation of Glutamatergic Receptor-Channels is Associated with Cross-Modal Reflexes Encoded in Barrel Cortex and Piriform Cortex

Jin-Hui Wang, Na Chen, Zilong Gao, Bo Wen, Chanfeng Chen, Yahui Liu.
Institute of Biophysics, Chinese Academy of Sciences, Beijing, China.

Associative learning is essential for cognitions. To better understand the mechanisms underlying associative learning, we examined whether the association of two signals induces a process that one signal evokes a recall of another signal, or turned around, and how glutamatergic receptor-channels are regulated to be involved in this reciprocal information retrieval (cross-modal reflex). In our mouse model, the two sensory systems, whisker-to-barrel cortex and olfaction-to-piriform cortex, were associatively activated by simultaneously stimulating whiskers and olfaction. This training procedure for 2 weeks led to odorant-induced whisker motion and whisker-induced olfaction responses. After this cross-modal reflex onset, the barrel and piriform cortices connected each other. Local filed potentials *in vivo* showed that the neurons in both barrel cortex and piriform cortex turned into processing whisker signal and odor one, respectively. The activity patterns of these cortical neurons in response to whisker signal and odorant one were distinct. These results indicate that the associative activation of barrel cortex and piriform cortex makes network neurons being able to store both whisker and odor signals as well as to recognize their differences through distinct encodings. With this reciprocal information retrieval, either of two associated signals can induce two responsive behaviors for well-organized cognitions and responses to environmental alerts. In terms of synaptic plasticity, we found that excitatory postsynaptic currents mediated by ionotropic glutamatergic receptor-channels in pyramidal neurons were upregulated in their amplitudes and frequency. By genome-wide sequencing, we observed that the upregulations of neuropilin (NMDAR accessory unit), AMPAR1 precursor, neuroligin 1~3 and tyrosine kinase in these cortical areas were associated with the cross-modal reflex and glutamate receptor-channel upregulation. [Supported by National Basic Research Program (2013CB531304 and 2011CB504405) as well as Natural Science Foundation China (30990261 and 81171033) to JHW].

964-Pos Board B719

2-Photon Imaging of Excitatory Potentials in Dendritic Spines using Voltage-Sensitive Dyes

Erika A. Hoyos-Ramirez, Corey Acker, Ping Yan, Leslie Loew.
Cell Analysis and Modeling, University of Connecticut, Farmington, CT, USA.

We developed a 2-photon imaging system that allows us to simultaneously perform glutamate uncaging and voltage sensitive dye (VSD) imaging from dendritic spines, which are the postsynaptic targets of excitatory inputs. Using this system and the intracellular fluorinated VSD di-2-AN(F)EPPTEA (Yan et al, 2012), we were able to record MNI-glutamate uncaging evoked excitatory postsynaptic potentials (EPSPs) from single spine heads that resemble mEPSPs (<1 mV at the soma) in the basal dendrites of LV pyramidal neurons. Our data suggests that in the spine heads, these EPSPs do not exceed more than 25 mV, and are attenuated by a mean factor of ~20 when they reach the soma. Interestingly, we have found no correlation between the EPSP amplitude in the spines and at the soma, which could be due to variability in the spine neck resistances. Based on this, we combined these experiments with FRAP of Alexa488, in order to estimate the spine neck resistance from the spines where we measured the EPSPs amplitudes. The time constant of equilibration of the cytosolic Alexa488, combined with the spine head volume, can be used to estimate the spine neck resistance. We used an image processing algorithm to determine the spine head volume from the 3D fluorescence distribution of Alexa488; from this, we have estimated spine neck resistances that range between 75-500 MΩ. By measuring the EPSP from a neighboring spine, we show experimentally that a spine with a neck resistance of ~75 MΩ does not fully compartmentalize the EPSP, and it can be seen by a neighboring spine ~5 um away with an amplitude > 5mV. NIH grants R01 EB001963, P41 GM103313.

965-Pos Board B720

A Multifunctional Pipette for Localized Drug Administration to Brain Slices

Aikeremu Ahemaiti[1], Alar Ainla[1], Gavin D.M Jeffries[1], holger Wigström[2], Aldo Jesorka[1], Kent Jardemark[3].
[1]Chalmers University of Technology, Göteborg, Sweden, [2]University of Gothenburg, Göteborg, Sweden, [3]Karolinska Institutet, Stockholm, Sweden.

We have developed a superfusion method utilizing an open-volume microfluidic device for administration of pharmacologically active substances to selected areas in brain slices with high spatio-temporal resolution. The method consists of a hydrodynamically confined flow of the active chemical compound, which locally stimulates neurons in brain slices, applied in conjunction with electrophysiological recording techniques to analyze the response. The microfluidic device, which is a novel free-standing multifunctional pipette, allows diverse superfusion experiments, such as testing the effects of different concentrations of drugs or drug candidates on neurons in different cell layers with high positional accuracy, affecting only a small number of cells. We demonstrate herein the use of the method with electrophysiological recordings of pyramidal cells in hippocampal and prefrontal cortex brain slices from rats, determine the dependence of electric responses on the distance of the superfusion device from the recording site, document a multifold gain in solution exchange time as compared to whole slice perfusion, and show that the device is able to store and deliver up to four solutions in a series. Localized solution delivery by means of open-volume microfluidic technology also reduces reagent consumption and tissue culture expenses significantly, while allowing more data to be collected from a single tissue slice, thus reducing the number of laboratory animals to be sacrificed for a study.

Magnetic Resonance Spectroscopy and Imaging

966-Pos Board B721

Optical Magnetic Imaging with Nitrogen-Vacancy Centers in Diamond

Keigo Arai[1], Chinmay Belthangady[2], Huiliang Zhang[2], Stephen J. DeVience[2], David Le Sage[2], David R. Glenn[2], Linh M. Pham[2], Lilah Rahn-Lee[3], Mikhail D. Lukin[2], Amir Yacoby[2], Arash Komeili[3], Ronald L. Walsworth[2].
[1]Massachusetts Institute of Technology, Cambrige, MA, USA, [2]Harvard University, Cambrige, MA, USA, [3]University of California, Berkeley, Berkeley, CA, USA.

We present recent work on developing an optical magnetic imaging system using nitrogen-vacancy (NV) color centers in diamond. The NV center is a photostable and bio-compatible sensor that can be used for both DC and AC magnetic field detection. We applied this imaging system to study DC magnetic fields produced by living cells. For AC detection, the spatial resolution and magnetic field sensitivities are further improved by use of phase encoding technique, widely used in the conventional magnetic resonance imaging.

967-Pos Board B722

Probing the Structural Topology of a Membrane Peptide in Mechcanically Aligned Lipid Bilayers using Bifunctional Spin Labeling EPR Spectroscopy

Lauren M. Bottorf, Lishan Liu, Indra D. Sahu, Robert McCarrick, Gary A. Lorigan.
Chemistry and Biochemistry, Miami University, Oxford, OH, USA.

Electron Paramagnetic Resonance (EPR) spectroscopy coupled with site-directed spin labeling (SDSL) is a powerful structural biology tool for studying the structural and dynamic properties of peptides, proteins, and nucleic acids. The most commonly used spin label for SDSL is methanthiosufonate (MTSL), however the flexibility of this spin label can introduce greater uncertainties in the EPR measurements for determining structure, side chain orientations and backbone motion of membrane protein systems. Another spin label, 2,2,6,6-tetramethylpiperidine-1-oxyl-4-amino-4-carboxylic acid (TOAC) has been found to be a useful alternative given its rigid structure, however it is very challenging to introduce this spin label into biological protein systems. The goal of this research is to develop an improved biophysical method for studying the structural and dynamic properties of membrane proteins using EPR spectroscopy that will overcome the limitations associated with MTSL and TOAC. A recently discovered bifunctional spin label (BSL) 3,4-Bis-(methanethiosulfonylmethyl)-2,2,5,5-tetramethyl-2,5-dihydro-1H-pyrrol-1-yloxy, will be utilized. Fmoc solid phase peptide synthesis (SPPS) will be used to generate a double cysteine mutant of the 23 amino acid α-helical membrane peptide, AChR M2δ. Once labeled with BSL and incorporated into 1,2-Dimyristoyl-sn-Glycero-3-Phosphocholine (DMPC) bilayers, alignment techniques utilizing EPR spectroscopy will be performed to examine the structural topology. This study will provide a structural biology tool that can be used to obtain very accurate and precise EPR measurements to answer several structural and dynamics related questions on membrane protein systems.