by HMR 1098 at high (100 ∈μM) but not low (10 ∈μM) concentration. By contrast, in atrial myocytes, both spontaneously activated and diazoxide-activated K_{ATP} currents were effectively inhibited by 10 ∈μM HMR 1098. Consistent with this finding, HMR 1098 inhibits $^{86}Rb^{+}$ effluxes through Kir6.2/SUR1 more effectively than Kir6.2/SUR2A channels in COSm6 cells. In excised inside-out patches, HMR 1098 effectively inhibited Kir6.2/SUR1 channels as well as Kir6.2/SUR2A channels in the absence of nucleotides, but inhibited Kir6.2/SUR1 channels more effectively than Kir6.2/SUR2A channels in the presence of MgADP and MgATP (mimicking physiological stimulation). Finally, dose-dependent enhancement of insulin secretion from pancreatic islets confirms that HMR 1098 is an effective inhibitor of Kir6.2/SUR1-composed K_{ATP} channels, and is not specific for SUR2A-composed channels.

713-Pos

Epac-Mediated Mobilization of Intracellular Calcium in Vascular Myocytes and the Downstream Effects on Arterial K_{ATP} Channels

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Exchange proteins directly activated by cyclic AMP (Epac or cAMP-GEFs) are a family of novel cAMP-binding effector proteins [1]. Using the Epac-specific cAMP analogue 8-pCPT-2-*O*-Me-cAMP we show cAMP-mediated but PKA-independent mobilization of $Ca^{2+}{}_i$ within vascular myocytes and downstream effects that culminate in the inhibition of ATP-sensitive potassium (K_{ATP}) channels.

Application of 8-pCPT-2-*O*-Me-cAMP (5μM) caused a 41.6 ± 4.7% inhibition of pinacidil-evoked whole-cell K_{ATP} currents recorded in isolated rat aortic smooth muscle cells. Inclusion of the Ca^{2+} chelator BAPTA (20μM) in the pipette-filling solution reduced the inhibition to 8.7 ± 4.4%, consistent with the idea that Epac mediates its effects by elevating $[Ca^{2+}]_i$. In support of this, 8-pCPT-2-*O*-Me-cAMP (5μM) caused a transient 171.0 ± 18.0nM increase in $[Ca^{2+}]_i$ in Fura-2-loaded myocytes, which persisted in the absence of extracellular Ca^{2+}. Caffeine-induced Ca^{2+} transients triggered in the presence of 8-pCPT-2-*O*-Me-cAMP typically showed a secondary Ca^{2+} increase, reminiscent of ectopic Ca^{2+} transients observed in Epac-activated cardiac myocytes [2]. While Ca^{2+} transients returned to baseline after 15-20s, the inhibition of K_{ATP} current was sustained, suggesting that Ca^{2+}*per se* does not affect channel activity and implicating the involvement of Ca^{2+}-activated enzymes. Preincubation with calcineurin inhibitors cyclosporin A (10μM) and ascomycin (5μM), significantly reduced the ability of 8-pCPT-2-*O*-Me-cAMP to inhibit K_{ATP} currents (inhibition 10.8 ± 2.8% and 7.3 ± 1.6%).

These findings suggest cAMP-mediated Epac activation in vascular smooth muscle mobilizes Ca^{2+} from internal stores and inhibits K_{ATP} channels through the activation of the Ca^{2+}-sensitive enzyme, calcineurin.

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Neuronal Systems & Modeling

714-Pos

Membrane Potential Imaging in Neurons using Fluorinated Voltage-Sensitive Dyes and a Custom Multiphoton Brain Slice Microscope

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In order to fully understand the physiology of fundamental neurophysiological processes such as synaptic integration and synaptic plasticity, direct recording of changes in membrane potential neuronal dendrites and spines is essential. In an effort to improve voltage-sensitive dye measurements of synaptic potentials and backpropagating action potentials, our group has developed new fluorinated dyes with enhanced photostability. We have also made performance improvements on our custom, non-linear optical microscope for greater sensitivity. By modifying a commercial Zeiss microscope we have added two ''up front'' epifluorescence detection channels and one transfluorescence detection channel. Optics for these new light paths were optimized using numerical ray tracing. Here we show that we are able to fill individual neurons with these dyes via somatic patch pipettes and record membrane potential changes in the soma and dendrites of Purkinje neurons in cerebellar brain slices. Using voltage clamp protocols, membrane potential was changed in a stepwise fashion, resulting in changes in membrane fluorescence. When excited with 1060 nm light, the new dyes typically produced changes in fluorescence (dF/F) between 3 and 7 % for 50 mV changes in membrane potential. Feasibility of using second harmonic generation to record membrane potential with these dyes was investigated in a cultured cell line by measuring dSHG/SHG, kinetics, and intensity as a function of dye concentration.

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715-Pos

Two-Photon Optogenetic Control of Neuronal Activity with Single Synapse Precision by Sculpted Light

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Recent advances in optogenetic techniques have provided new tools for controlling neuronal activity, opening up the way to a range of studies in neuroscience. The most widely used approach has been the optical activation of the genetically expressed light-gated ion channel Channelrhodopsin-2 (ChR2) to initiate population activity in neuronal circuits. However, single cell resolution of optogenetic activation has remained challenging. This is because neither single-photon nor conventional two-photon excitation provides the necessary combination of high spatial selectivity and the simultaneous stimulation of a sufficiently large membrane area necessary to induce fast and significant depolarizations by ChR2 in a single neuron.

The presented work reports on two-photon excitation of ChR2 allowing the generation of fast and large ChR2-mediated currents in single cells with high spatial and temporal resolution by using temporally focused beams. It is demonstrated that this technique efficiently induces strong depolarization and reliable action potential firing in single ChR2-expressing neurons in rat and mouse hippocampal slices. It is further shown that subcellular compartments such as dendrites and large presynaptic terminals can be activated by the TF-2P technique. The superb spatial and temporal resolution provided by this technique allows so far unattainable precision for fine manipulation of neuronal activity to study and control the function of neuronal microcircuits *in vitro* and *in vivo.*

716-Pos

Combining Optical Tweezers, Laser Microdissectors and Multichannel Electrophysiology for the Non-Invasive Tracing and Manipulation of Neural Activity on Single Cell and Network Level

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During differentiation, cell processes initiate exploratory motion to create connections with other cells thereby creating a tissue architecture that is capable of performing complex tasks. The interplay between mechanical and chemical stimuli seems necessary for triggering the proper biochemical reactions that eventually lead to the functional organization of cells and tissue[1].

There are different approaches for studying the focused mechanical-chemical transduction, either at single cell[2] level or at tissue[3] level. To better understand tissue development (cell differentiation, cells contact formation, tissue organization), we would like to bridge the gap between experiments on single cells and complex tissues. Therefore we are developing a system for combining optical techniques such as optical tweezers[4] and a laser dissector with electrophysiological tools. Optical tweezers permit to apply localized mechanochemical stimuli onto cells[5] while a laser dissector can alter individual neuronal connections[6]. By adopting neuronal networks as a biological model, neural signal transmission affected by such external stimuli can be recorded non-invasively by microelectrode arrays.

Ongoing work is targeted at correlating the temporary or lasting changes in neural networks to the type and site of the stimulus.

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717-Pos

Astrocytic Processes Ensheathing Synaptic Glomeruli cause Anomalous Extracellular Diffusion

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Brain extracellular space forms a channel where signaling molecules, growth factors and therapeutics diffuse. Characterization and quantification of the extracellular diffusion is thus important for intercellular signaling and drug delivery. We have recently reported that the extracellular diffusion is *anomalous* in the granular layer of rat cerebellar slices (anomalous diffusion model quantified an average anomalous exponent d_w 5.0). In this respect, the granular layer significantly differs from most brain regions where the extracellular diffusion is *normal* (i.e., $d_w \approx 2.0$). In biological systems, anomalous diffusion may

be caused by concave geometrical formations that transiently trap diffusing particles. In rat granular layer, large and abundant synaptic glomeruli formed by concave processes of astrocytes that ensheathe complex synapses may function as the traps. To test this hypothesis, we employed Integrative Optical Imaging method to measure diffusion of small dextran molecules (*MW* 3000) in the granular layer of isolated turtle cerebellum where complex synapses are present but have no or very limited astrocytic ensheathment. In turtle granular layer, the anomalous diffusion model quantified $d_w = 2.6$, which is significantly less than $d_w = 5.0$ observed in rat granular layer but similar to d_w extracted from normal diffusion in a free medium (2.1) and in other brain regions (e.g., 2.3 in rat neocortex). Thus the extracellular diffusion was normal in the granular layer of turtle cerebellum that lacks trap-like astrocytic ensheathment of synaptic glomeruli. In conclusion, diffusion measurements in turtle support our hypothesis that astrocytic processes ensheathing synaptic glomeruli cause anomalous extracellular diffusion. Our approach is potentially useful for quantification of astrocytic ensheathment in the living brain under normal and pathological conditions. Our result also has implications for the interstitial transport of molecules and drugs in brain regions containing synaptic glomeruli. Supported by NIH(NS047557).

718-Pos

A Model of Spike-Timing-Dependent Plasticity

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It has been shown that postsynaptic calcium dynamics plays an important role in spike timing-dependent plasticity (STDP), a process in which changes in synaptic strength are determined by the relative timing of pre- and postsynaptic activity. It has been suggested STDP involves a postsynaptic chemical network with stable states corresponding to long term potentiation (LTP) and long term depression (LTD). It is believed that the switching of this network between these states is driven by the postsynaptic Ca2+ concentration, but the manner in which the Ca2+ dynamics causes the switching to depend on the relative timing of pre- and postsynaptic activity remains unclear. We describe a model of STDP that combines the chemical network model of Pi and Lisman (2008), with a model of Ca2+ dynamics that builds on the work of Shouval and coworkers (2002). Following Shouval and coworkers, we assume that a portion of the influx of postsynaptic Ca2+ is controlled by NMDA receptors that allow an inward Ca2+ current in response to both glutamate binding and a back propagating action potential (BPAP). To this we add a contribution from voltage dependent calcium channels (VDCCs). We show that this model is able to reproduce the observed time dependence of STDP when a single presynaptic pulse is either followed or preceded by a single BPAP. The behavior of the model with triplet pulse protocols, e.g., two presynaptic pulses separated by a BPAP, or two BPAPs separated by a presynaptic pulse, and the incorporation of more sophisticated models of AMPA trafficking are also described.

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719-Pos

Plasticity of Chloride Homeostasis can Cause Bistability and a Switch in Neuronal Spiking Pattern

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Plasticity of $GABA_A$/glycine synapses can be mediated by changes in intracellular chloride concentration ($[Cl^-]_i$). While chloride influx through ligand-gated channels alters $[Cl^-]_i$, the extent and time scale of those changes depend on transmembrane cation-chloride cotransporters (CCC). Because changes in the chloride reversal potential influence the membrane potential which in turn affects chloride currents, we show that there is a positive feedback loop between intracellular chloride accumulation, excitation and firing. To investigate further how this positive feedback loop modifies the firing dynamics of the cell, we extended the Morris-Lecar model with an extra dimension to account for changes in $[Cl^-]_i$. The model exhibits a novel form of chloride-related bistability and associated hysteresis which can switch the neuron from type I to type II firing. A 2D reduction of the model allowed us to identify regions in parameter space for which this change in firing type occurs. Chloride-related bistability is most likely to occur when CCC activity is reduced. This phenomenon can dramatically prolong changes in $[Cl^-]_i$ caused by an otherwise brief trigger. We quantified how chloride-mediated $GABA_A$ synaptic plasticity allows the spiking pattern to carry information about past inhibitory activity. This is achieved by computing the mutual information between spiking and past inhibitory activity for various time windows. While high levels of CCC activity limits $[Cl^-]_i$ mediated ionic plasticity, reduced CCC activity decreases the mutual information between inhibitory input and spiking output. Given this trade off, there is an optimal level of CCC activity for which mutual information is maximal given different input distributions and different time windows. These results show that beyond membrane currents taken into account in conventional cable modeling, chloride dynamics and electroneutral transport activities critically determine the computational properties of neurons.

720-Pos

Integration of Cellular Metabolism and Membrane Excitability in Cerebellar Purkinje Neurons

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Calcium and membrane physiology are crucial to cerebellar Purkinje neuron function. Purkinje cell physiology involves phosphoinositide/calcium signaling and calcium influx through voltage-gated calcium channels, as well as potassium efflux through (i) voltage-gated, and (ii) calcium-activated voltage-gated channels (primarily the BK channel). The interaction between phosphoinositide-induced calcium signaling and calcium-activated/voltage-gated potassium channels have not been explored extensively. We have developed computational models to explore the integration of these mechanisms. We used an electrophysiological model in NEURON to parameterize a compartmental Virtual Cell (VCell) model. We then combined our published calcium metabolism model (created in VCell) with the electrophysiological model. We investigated the influence of IP3R1-mediated calcium release from smooth endoplasmic reticulum close to the plasma membrane on the activity of the BK channel and thus on membrane potential. The model predicts that supralinear IP3R1-mediated calcium release into a submembrane shell can activate BK channels. When coupled with synaptic conductance changes, this activation of the BK channels increases the rate of repolarization (RR) of the spine. As the voltage changes in the spine propagate to the soma, the corresponding RR in the soma is also increased. Simulation of IP3R1 ko abolishes any increase in the RR in both the spine and the soma. Reduced IP3R1 abundance (as found in some cerebellar ataxias; in model, 10% - 50% of the original value), almost completely abolishes any increase in the RR, in both spine and soma. Increasing the sensitivity of IP3R1 to IP3 restores normal IP3R1-mediated calcium, and restores increased activation of the BK channels. The resulting RR of both the spine and the soma are also restored. These results suggest that the BK channel may play a role in integrating signals from cellular metabolism and membrane excitability. (Supported by NIH P41 RR013186)

721-Pos

The Role of Phosphorylation on Mouse Neurofilament Medium Protein (NF-M) Sidearms

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Neurofilaments (NF) are important cytoskeletal filaments that are assembled from three distinct molecular weight proteins - neurofilament light (NF-L), medium (NF-M), and heavy (NF-H). The three proteins are bound to each other laterally forming 10-nm filamentous rods along with sidearm extensions that belong to the C-terminal tails of the proteins. These tails vary in number and sequences of their amino acid residues, and are abundant with charges. Additionally, the sidearm polypeptides attain negative charges through serine phosphorylation of the Lys-Ser-Pro (KSP) repeat motifs that are particularly found in NF-H and NF-M sidearms. NF protrusions mediate the interaction between neighboring filaments, and maintain axonal diameter. However, the role of individual NF proteins and their phosphorylations in regulating interfilament distances, and hence axonal diameter, is not fully understood. A number of studies have implicated NF-M proteins as critical in regulating axonal caliber. However, the conventional viewpoint that NF-M phosphorylation increases axonal caliber has been challenged by recent experimental study that disputes the effect of NF-M phosphorylation in modifying axonal caliber (Garcia et al. J. Neurosci. (2009) 29: 1277-1284). By employing gene replacement technique, the authors deduced that phosphorylation of NF-M KSP repeat is not required for myelin-dependent radial axonal growth. To better understand the effect of NF-M phosphorylation, we investigated the structural organization of mouse NF under phosphorylated and dephosphorylated conditions. We employed the 3D sequence-based coarse-grained model of NF brush (Chang et. al., J. Mol. Biol. (2009) 391:648-660) to perform Monte Carlo simulations of mouse NF by using the sequence and the stoichiometry of mouse NF proteins. Our result shows that the phosphorylation of mouse NF-M does not change the radial extension of NF-M, supporting the notion that NF-M phosphorylation has no effect on axonal diameter of mouse.