760-Pos Board B540
Imaging Calcium Activity Patterns in the Drosophila Pupal Ecdysis Neural Circuit
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Insects execute a behavioral program, called an ecdysis sequence, to shed their cuticle at each developmental molt. The neural circuit controlling the ecdysis sequence consists of approximately 300 peptidergic neurons that express the Ecdysis Triggering Hormone receptor (ETHR) and are activated by peripheral release of Ecdysis Triggering Hormone (ETH). While several groups of neurons within the network play known roles in the ecdysis sequence, such as those expressing the hormones CCAP and bursicon, the timing of their activity is poorly understood and likely involves regulation by as yet unidentified inhibitory neurons. In general, the patterns of activity within the ecdysis network and how they give rise to motor output remains enigmatic. Using the genetically tractable Drosophila model system, and focusing on the ecdysis sequence executed at the pupal molt, we have investigated the evolution of network activity using Gal4 driver lines to express UAS-RCaMP or UAS-GCaMP6 Ca^{2+} biosensors in subsets of neurons in the ETHR network or its downstream motor neurons. We are able to elicit the ecdysis sequence in an excised pupal brain by application of exogenous ETH and visualize the neural activity underlying the ecdysis-specific motor patterns in targeted neurons. The quantitative temporal information provided by Ca^{2+} biosensors, when combined with anatomical data derived from structured illumination and diffraction-limited fluorescence microscopy techniques, provides insights into how the activity patterns of the ETHR circuit are generated.

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The Influence of Voltage Sensor Activity on Arclight Dynamics
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Many genetically-encoded voltage indicators have been developed based on voltage-sensitive phosphatases. One of the most promising of these indicators is Arclight, largely due to its substantial signal in response to voltage changes. Although it is evident that the fluorophore is fundamentally following the voltage sensor movement, the fluorescence change is comprised of multiple components; how these fluorescence components reflect voltage sensor movement remains poorly understood. Using simultaneous electrophysiological and optical recordings of oocytes expressing wild-type and mutant Arclight constructs, we investigated the influence of altered voltage sensor behavior on Arclight fluorescence. We found that the latency before fluorescence change onset correlated with gating charge movement, and that the fluorescence change following prolonged depolarizations showed alterations in kinetics and thermodynamics consistent with voltage sensor relaxation. Other features of fluorophore movement, however, were less obviously coupled to voltage sensor dynamics. These results may help guide future attempts to optimize the properties of Arclight and related fluorescent proteins. Support: NIH GM030376.

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Nonlinear AMPA Sensitivity to Glutamate and Recruitment of NMDA Activity in Dendritic Spines
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Our lab has been developing and improving the optical and molecular tools and techniques to record EPSPs (excitatory postsynaptic potentials) in dendritic spines. With a dual laser 2-photon microscope we are able to record from voltage-sensitive dye (VSD) labeled spines from cortical pyramidal neurons while evoking unitary EPSPs using glutamate uncaging. By varying the uncaging laser intensity we observe a nonlinear response to glutamate when the resulting EPSPs amplitudes are observed at the soma. By optically recording EPSPs in spines we can determine that the observed nonlinearity is often present even when spine EPSPs are relatively small, i.e. < 10 mV. Pharmacology is used to block NMDA receptors and determine the recruitment of these receptors and their contribution to both voltage and calcium influx. Calcium imaging reveals that NMDA receptors are recruited in a very linear fashion according to the spine depolarization. This calcium influx however, does not appear to play a significant role in the spine depolarization. Finally, modeling is used to reconcile the observed phenomena with detailed models of AMPA and NMDA receptor gating, and glutamate binding.

763-Pos Board B543
Biophysical Approaches to the Study of Traumatic Brain Injury
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We applied biophysical methods to study the anatomical origins of diffuse axonal injury (DAI), a common pathological finding in closed_head traumatic brain injury. The only method capable of detecting DAI non-invasively is diffusion tensor imaging (DTI), which is sensitive to the anisotropic movement of water along axons. Regions in the white matter of the brain exhibiting abnormally low fractional anisotropy are indicative of axonal disruption and are clinically interpreted as evidence of DAI. However, a direct relationship between reduced fractional anisotropy and anatomic axonal injury has not been firmly established. In this study, regions in the corpus callosum with reduced fractional anisotropy were identified in DTI maps of post-mortem brains imaged on a 9.4-Tesla magnetic resonance microscope. The anatomic structures of the corpus callosum in regions with normal and reduced fractional anisotropy were subsequently examined by Coherent Anti-Stokes Raman Scattering (CARS) microscopy using the symmetric C-H stretching band at 2845 wavenumbers to highlight the myelin membrane. Regions of the corpus callosum with normal fractional anisotropy revealed intact axons running in orderly parallel tracks. In contrast, regions with low fractional anisotropy exhibited bisected axons, axonal fragments, regions devoid of myelin lipid, and a loss of parallel axonal orientation. The results of this study establish an anatomical link between DTI and DAI that may result in improved methods to detect, study, and treat traumatic brain injury.

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Hypotheses for Hair Cell Ribbon Synapses Probed by a Biomimetic System
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The conversion of sound waves to electrical impulse in the auditory nerve of the inner ear takes place in the organ of Corti through the process of synaptic transmission in and around the ribbon synapse. Finding the relation between the functional and physiological characteristics of the ribbon synapse is one of the central issues in auditory science nowadays. There has been a hypothesis on inner hair cells (IHC) that complex broad band sounds can be encoded by multiple ribbon synapses in an IHC. We provide a hypothesis on the spectral analysis by multiple ribbon synapses, where heterogenous glutamate release rates in a IHC is the key to the spectral analysis. To demonstrate our hypothesis, we fabricate a neuromorphic device that models the hair cell bundle and its afferent neuron, and investigate its response to pure-tone and human voice sounds. Our biomimetic experimental results go beyond theoretical simulation While mechanical oscillators' velocity dependence of relaxation and noise can not but be assumed theoretically, our biomimetic approach goes beyond this limitation of such assumption and is self-consistent by its own. We observe that for the neuronal spike rates to mimic the voice sound signal of a word, a distribution of the spontaneous neurotransmitter relaxation rates is necessary. We also discuss a physical microscopic model for the ribbon synapse which is based on charge transport theory.

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The Energetics of High Frequency Discharge in Electrocytes: A Mathematical Model with Explicit Pumps
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In weakly electric fish, continual high frequency action potential (AP) discharge from electrocytes allows for sensing of the physical environment and communication among conspecifics. AP-related costs arise directly from activity of the Na/K-ATPase as it uses ATP to maintain appropriate levels of $[Na_i]$ and $[K_o]$, thereby recharging the cellular batteries for ongoing AP-discharge. Recent findings from respirometry (during high-frequency signaling over a range of frequencies) plus electrophysiology and modeling indicate that for high discharge rates a trade-off in efficiency occurs (Lewis et al 2014 J Neurosci 34:197). Electrocyte repolarization depends on a delayed rectifier whose open probability is modulated by $[Na_i]$, and also by an inward rectifier. The biophysical model used did not, however, include explicit Na/K pumping ($d[Na_i]/dt$ instead depended on persistent I_{Na} and a fractional pumping rate for Na^+ ions). We now compare the computational outcomes for the first model with computed behaviors obtained when a Lauger type Na/K-ATPase pump is used to describe the regulation of Na^+ and K^+ gradients. This makes it straightforward, for instance, to bypass the previous simplifying (but perhaps