pacing conditions, the model predicts that the Na+ channel blocker flecainide results in development of tachyarrhythmias. However, the same drug is predicted to exhibit antiarrhythmic effects on slow heart rate dependent arrhythmia triggers. Our results suggest that drug efficacy in arrhythmia treatment is drug and circumstance dependent and that a computational approach can be utilized to predict experimentally testable outcomes.

1109-Symp
Optogenetics: Development And Application
Karl Deisseroth.
Stanford University, Stanford, CA, USA.
Integrating microbial opsins and solid-state optics allows millisecond-precision bidirectional control of defined cell types in freely behaving mammals. Following the introduction of the microbial opsin genes ChR2 and NpHR to neurobiology, genomic strategies allowed the discovery and adaptation for neuroscience of a third major optogenetic tool, namely a cation channel (VChR1) with action spectrum significantly redshifted relative to ChR2, to allow tests of the combinatorial interaction of cell types in circuit computation or behavior. We also have developed genetic targeting tools for versatile use of microbial opsins with existing resources including cell type-specific promoter fragments or Cre-LoxP mouse driver lines suitable for a wide variety of neuroscience investigations, and developed integrated fiberoptic and solid-state optical approaches to provide the complementary technology to allow specific cell types, even deep within the brain, to be controlled in freely behaving mammals.

1110-Symp
Semiconductor Chips with Nerve Cells and Brain Tissue
Peter Fromherz.
Max Planck Inst Biochem, Martinsried, Germany.
The lecture describes the electrical interfacing of semiconductor devices with cultured neuronal networks and brain tissue in both directions. Individual capacitors and transistors on silicon chips are used as well as multi-transistor and multi-capacitor arrays fabricated by an extended CMOS technology [1]. On the biological side, three levels of interfacing are considered with recombinant ion channels, with individual nerve cells from snails and rats, as well as with rabbit retinae and slices from rat hippocampus. Particular attention is given to the mechanism of signal transduction between the electronic and ionic system that avoids electron exchange between semiconductor and electrolyte. In that respect, the Johnson noise is most useful tool to determine the seal resistance of cell-chip junctions. In the case of electronic stimulation of neuronal activity, a displacement current across electrolyte-oxide-semiconductor (EOS) capacitors gives rise an Ohmic current along the seal resistance and to a voltage change across the cell membrane that opens ion channels. In the case of electronic recording of neuronal activity, the current through ion channels gives rise to an extracellular voltage in the cell-chip junction that plays the role of a gate voltage on EOS field-effect transistors. In general, the interfacing of nerve cells from invertebrates (leech, snail) is simpler to achieve than the stimulation and recording of mammalian neurons because the capacitive and ionic currents are larger for larger cells. With cultured and acute brain slices, the interfacing refers to small groups of neurons. The implementation and understanding of neuro-electronic interfacing is a basis for applications in biosensorics, neurophysiology, neuroprosthetics and experiments that may lead to neurocomputers.
[1] P. Fromherz, Solid State Electronics 52 (2008) 1364.

Minisymposium 2: Microtubular Motors: Structural and Functional Diversity

1111-MiniSymp
Direct Observation of Individual Kinesin Head Motions
Nicholas R. Guydosh, Steven M. Block.
Stanford University, Stanford, CA, USA.
Optical-trapping assays for kinesin typically involve attaching a bead to the common stalk of the protein, whose motions report the average position of the molecule. Individual displacements produced by the separate heads therefore remain unresolved. We developed a novel assay for tracking a single head of Kinesin-1 while under controlled loads, by attaching a bead to one of the two head domains via a short (70 bp) DNA tether. This assay can directly report binding of the tethered head to the microtubule. Under hindering loads, we observed steps of ~16 nm, as anticipated for heads moving in a hand-over-hand walk. Under assisting loads, we observed large jumps (>16-nm) in displacement at the start of step dwells, as load pulled the rear head forward beyond its partner head by ~4 nm. Torque generated between the points of head attachment of the DNA and the neck linker tends to rotate the head, explaining this 'overshoot' feature. The durations of overshoots depend on the ATP concentration, implying that ATP binding to the new rear head allows the front (DNA-linked) head to rotate back to its normal orientation and bind the microtubule. To directly test whether one head is free to diffuse about its bound partner head between steps, we applied rapidly oscillating (hindering and assisting) loads to kinesin during stepping and measured the time-dependent difference between forward and rearward displacements of the bead. The magnitude of this signal varied between two discrete values, corresponding to those intervals when the bead-linked head adopted bound and unbound states. The existence of an unbound state disfavors models where one head docks against its partner between steps. We conclude that internal strain, generated whenever both heads bind the microtubule, is responsible for gating the kinetic cycle to ensure kinesin processivity.

1112-MiniSymp
The ATP State of a Mitotic Kinesin-5 Bound to Microtubules
Andrew Bodey[1], Masahide Kikkawa[2], **Carolyn Moores[1]**.
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The mitotic spindle is essential for faithful cell division. It is built from microtubules and is orchestrated by many proteins, including members of the kinesin superfamily. Kinesin-5 motors are essential for mitosis in many organisms and are involved in formation and maintenance of spindle bipolarity. Kinesin-5s share some properties with other kinesins including the ability to move - albeit slowly - towards the plus ends of microtubules. However, kinesin-5s have a number of unique properties, and are also of interest for cancer treatment because kinesin-5-specific small molecule inhibitors have been identified and are in clinical trials.
Outstanding mechanistic questions about kinesin-5 motors relate to their interaction with microtubules. We set out to understand this interaction using cryo-electron microscopy and image processing. Cryo-electron microscopy is uniquely suited to this goal since microtubules are too large and heterogeneous to be studied by other structural techniques. Using the motor domain from Klp61f (the Drosophila kinesin-5), we imaged microtubules bound by the motor in an ATP-like state and calculated the structure of the complex at ~10Å resolution. At this resolution, we are able to see the density associated with most α-helices in both the motor and the microtubule and visualise the motor in a tight-binding, AMPPNP conformation. The docked tubulin structure shows an excellent fit to our map, but available kinesin-5 crystal structures do not match the conformation of the motor in our maps, indicating that microtubule binding induces a conformational change in the kinesin-5 motor. Thus, calculation of kinesin-microtubule structures are essential for revealing the precise mechanism by which motors use energy from ATP and microtubule binding to generate force. Our structure also provides insight into the mechanisms by which anti-cancer drugs elicit their therapeutic effect.

1113-MiniSymp
LIS1 Converts Dynein to a Persistent-force State: A Molecular Model to Explain Lissencephaly
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Cytoplasmic dynein is involved in diverse tasks such as cargo transport along microtubules, cell division and nuclear migration. We have used biochemical and biophysical tools to examine the regulatory roles of two proteins which are crucial parts of the dynein pathway: NudE and LIS1. LIS1 is the causative gene for the developmental brain disease, lissencephaly, which is associated with dynein-dependent defects in cell migration and division. NudE is a dynein-LIS1-interacting protein also implicated in brain development and mitosis. Using laser trapping of beads coated with dynein and its associated factors at the single-molecule limit we find that NudE reduces the frequency of motor-microtubule binding events and inhibits motor travel. In contrast, LIS1 dramatically extends dynein's force generating state. LIS1 alone binds transiently to dynein, but NudE recruits LIS1 to form a stable ternary complex with persistent force-generating activity. These results have important biological implications. LIS1 is known to participate in dynein functions that involve transport of very high loads, such as nuclear movement through the embryonic brain (Tsai, Bremner, Vallee, 2007, Nat. Neurosci. 10:970). Our results strongly suggest that it is this aspect of LIS1 function that makes the developing brain uniquely sensitive to decreased LIS1 expression in human lissencephaly. Supp. by grants GM47434, HD40182, and GM070676.