which will determine the broader applicability of this technique.

30. Relationship Between Different Short-Term Plasticity Mechanisms at an Identified Synapse.
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Short-term plasticity changes the amount of neurotransmitter release and thus the amplitude of postsynaptic potentials (PSP) at chemical synapses, depending on the stimulation frequency. An increase in the PSP amplitude, known as facilitation, is caused by an increase of the release probability due to presynaptic calcium accumulation. A decrease in the PSP amplitude after repetitive stimulation, known as depression, is attributed to presynaptic vesicular depletion. At some synapses depression occurs upon pairs of impulses at low frequencies, while paired impulses at higher frequencies produce facilitation, which is not congruent with the hypothesis of vesicular depletion. Here, we investigated the mechanisms that produce this type of depression at the synapse between pressure-sensitive and anterior pagoda neurons of the leech central nervous system. Presynaptic impulses separated by variable intervals were produced, and plasticity was analyzed by comparing the amplitudes of the resulting PSP. Two presynaptic impulses separated by 200 ms produced facilitation in the second PSP, which gradually decreased as the interval increased. Intervals larger than 800 ms produced depression. Increasing the initial transmitter release by rising extracellular calcium or by producing more impulses increased depression, suggesting a mechanism mediated by vesicular depletion. However, facilitation could be produced after a depressed PSP, indicating that there are enough synaptic vesicles at the terminal to sustain release and suggesting an additional depression mechanism, which depends on the interval between impulses. A plasticity mathematical model that considers vesicular depletion and changes in release probability due to intracellular calcium reproduces facilitation after short intervals, but not depression after long intervals, showing that known mechanisms do not explain this plasticity phenomenon. We propose that, in addition to vesicular depletion, there is a mechanism acting on vesicle availability whereby an impulse prepares vesicles to fuse with the membrane, but this is reversed after 800 ms, preventing their fusion.

31. Monitoring Voltage Fluctuations of Internal Cell Membranes.
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In eukaryotic cells, the endoplasmic reticulum (ER) is the largest continuous membrane-enclosed network that surrounds a single lumen. Using a new genetically encoded voltage indicator (GEVI), we applied the patch clamp technique to HEK293 cells and found that there is a very fast electrical interaction between the plasma membrane and internal membrane(s). This discovery suggests a novel mechanism for interaction between the external membrane and internal membranes as well as an additional mechanism for interactions between the various internal membranes. The ER may transfer electrical signals between the plasma membrane, the Golgi apparatus, the nuclear envelope, the mitochondria, and other internal organelles. The internal membrane signal is reversed in polarity but has a time course similar to that of the plasma membrane signal. The optical signal of the GEVI in the plasma membrane is consistent from trial to trial. However, the internal signal decreases in size with repeated trials. This dynamic behavior of the internal signal suggests that voltage may stress internal membranes causing them to remodel and/or change their resistance.

32. Nicotine in the Endoplasmic Reticulum.
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Nicotine activates plasma membrane (PM) nicotinic receptors (nAChRs), but also permeates into the endoplasmic reticulum (ER) and *cis*-Golgi, and there binds to nascent nAChRs. Other psychiatric and abused drugs may also enter the ER and bind their classical targets. Further progress requires direct proof, quantification, and time resolution of these processes in live cells and in the brain of animals. Therefore, we are developing genetically encoded fluorescent biosensors to study the subcellular pharmacokinetics of neural drugs.

OpuBC, a monomeric bacterial periplasmic binding protein (PBP), has (a) a binding site for amines including a cation-π box and (b) ligand-induced "Venus flytrap" conformational change involving relative motions of two domains—features reminiscent of Cys-loop receptors. We insert circularly permuted "superfolder" GFP (cpGFP), flanked by several-residue linkers, within inter-domain hinge regions. We apply directed evolution, including X-ray crystallography, to optimize the

sensing of drugs, and have achieved the goal of ΔF/F0 > 1 at 1 μM for several drug-biosensor pairs.

Our most detailed studies use "intensity-based nicotine-sensing fluorescent reporters" (iNicSnFRs). We insert targeting and retention sequences to direct the constructs to the ER or to the PM of clonal mammalian lines and cultured neurons. Live-cell video imaging shows that, after we jump [nicotine] (increase or decrease) near cells, the drug appears in (or leaves) the ER within <10 s. Responses are robust, even at [nicotine] in the brain of a smoker. When extrapolated to human smoking and vaping, these data explain aspects of nicotine addiction. Kinetics with varenicline are nearly as fast, rationalizing its action in smoking cessation.

Other iDrugSnFRs detect antidepressants, opioids, and antipsychotics. We hope that further application of these tools will show the initial steps in the pathway of pharmacological chaperoning and its sequelae—aspects of "inside-out" neuropharmacology.

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33. Imaging Endogenous Neuronal Kv2 Potassium Channel Gating With a Fluorescent Tarantula Toxin. ROBERT G. STEWART,[1] PARASHAR THAPA,[1] REBECKA SEPELA,[1] OSCAR VIVAS,[1,2] LAXMI K. PARAJULI,[2] MARK W. LILLYA,[1] SEBASTIAN FLETCHER-TAYLOR,[1] BRUCE E. COHEN,[3] KAREN ZITO,[2] and JON T. SACK,[1] *[1]Physiology and Membrane Biology, University of California, Davis; [2]Center for Neuroscience, University of California, Davis; [3]Biological Nanostructures Facility, Laurence Berkeley National Laboratory*

Neurons display diverse electrical signaling patterns throughout the nervous system. This electrical signaling is generated by voltage-gated ion channel subtypes working in concert. The localization and gating of channel subtypes determine how they contribute to neuronal signaling. However, it is challenging to establish where endogenous channel subtypes are expressed in live tissue and how active the channels are. We have generated molecular probes that optically report changes in the voltage activation status of Kv2 ion channels. These probes were developed from the tarantula peptide guangixitoxin (GxTX) that binds to resting Kv2 ion channels and disassociates when channels are activated by voltage. When GxTX is conjugated to fluorescent dyes, binding and disassociation can be visualized with fluorescence microscopy. By monitoring changes in fluorescence of a GxTX variant with 2-photon microscopy, we can observe voltage activation of endogenous Kv2 channels in CA1 neurons in rat hippocampal slices. These voltage-dependent changes from neurons are consistent with GxTX fluorescence responses from heterologously expressed Kv2.1 channels. The GxTX probe appears selective for Kv2 channels. In neurons and CHO cells, transfected with GFP-Kv2.1 or GFP-Kv2.2, GxTX puncta colocalize with GFP puncta. Colocalization with other GFP-tagged channel subtypes was not detected. Kv2 channel binding was retained in the presence of its neuronal auxiliary subunit AMIGO-1. We conclude that optical signals from conformation-selective ion channel probes can report when and where an endogenous ion channel subtype activates in individual neurons within a brain slice.

34. Defining Olfactory Bulb Functions via Comparison of Input and Output. DOUGLAS A. STORACE,[1] SHENG ZHONG,[1] and LAWRENCE B. COHEN,[1,2] *[1]Department of Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, CT 06520; [2]Center for Functional Connectomics, Korea Institute of Science and Technology, Seoul 136-791, Republic of Korea*

Humans and other animals can recognize an odorant as the same over a range of odorant concentrations. It remains unclear whether the olfactory bulb, the brain structure that mediates the first stage of olfactory information processing participates in generating this perceptual concentration invariance. Olfactory bulb glomeruli are regions of neuropil that contain input and output processes: olfactory receptor neuron nerve terminals (input) and mitral/tufted cell apical dendrites (output). Differences between the input and output of a brain region define the function(s) carried out by that region. We compared the activity signals from the input and output across a range of odorant concentrations. The output maps maintained a relatively stable representation of odor identity over the tested concentration range, even though the input maps and signals changed markedly. The results were similar when performing the measurements in the same preparation using different optical sensors, or in different preparations using the same sensor. These results provide direct evidence that the mammalian olfactory bulb likely participates in generating the perception of concentration invariance of odor quality. Our imaging methods should also be useful for determining the input/output transformation in other regions of the mammalian brain.

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35. A Second-Generation SCAPE System for Fast, 3D Imaging of Neural Activity. VENKATAKAUSHIK VOLETI, WENZE LI, KRIPA PATEL, MOHAMMED A. SHAIK, CITLALI PEREZ-CAMPOS, and ELIZABETH M.C. HILLMAN, *Departments of Biomedical Engineering and Radiology, Mortimer B. Zuckerman Mind Brain Behavior Institute, Columbia University in the City of New York, NY 10027*

Swept, confocally-aligned planar excitation (SCAPE) microscopy is a 3D, light-sheet imaging technique