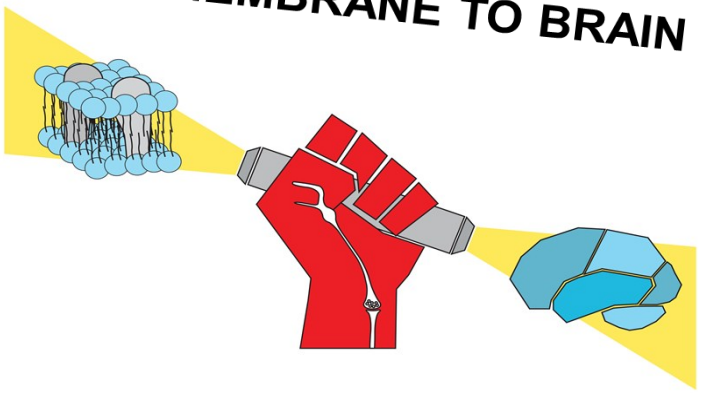
Society of General Physiologists

71st Annual Meeting and Symposium

6-10 September 2017

Marine Biological Laboratory

THE OPTICAL REVOLUTION IN PHYSIOLOGY: FROM MEMBRANE TO BRAIN



Organizers: Richard Kramer (University of California Berkeley)
and Edwin Levitan (University of Pittsburgh)

PROGRAM AND ABSTRACTS

2017 SGP Symposium Sponsors

The Society of General Physiologists would like to acknowledge the sponsors who have helped make this meeting such a prominent gathering of researchers.

Gold Sponsor

Leica MICROSYSTEMS

Silver Sponsors

3i Intelligent Imaging Innovations

Biotium

CHROMA®

Nikon

Bronze Sponsors

THORLABS

ZEISS

General Sponsor

SUTTER INSTRUMENT®

Funding for this conference was made possible (in part) by R13 grant NS103423 from the National Institute Of Neurological Disorders And Stroke (NINDS) and the National Center For Advancing Translational Sciences (NCATS). The views expressed in written conference materials or publications and by speakers and moderators do not necessarily reflect the official policies of the Department of Health and Human Services; nor does mention of trade names, commercial pratices, or organizations imply endorsement by the U.S. Government.

2017 SGP Symposium Major Supportors

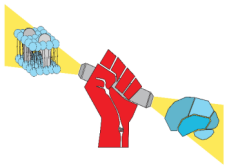
The Society of General Physiologists would like to acknowledge our major supporters who have helped make this meeting such a prominent gathering of researchers.

University of Pittsburgh

We are grateful for the generous institutional contribution for supporting junior scientist awards from the University of Pittsburgh by Dr. Arthur Levine, Dean of the School of Medicine and Senior Vice Chancellor of the Health Sciences.

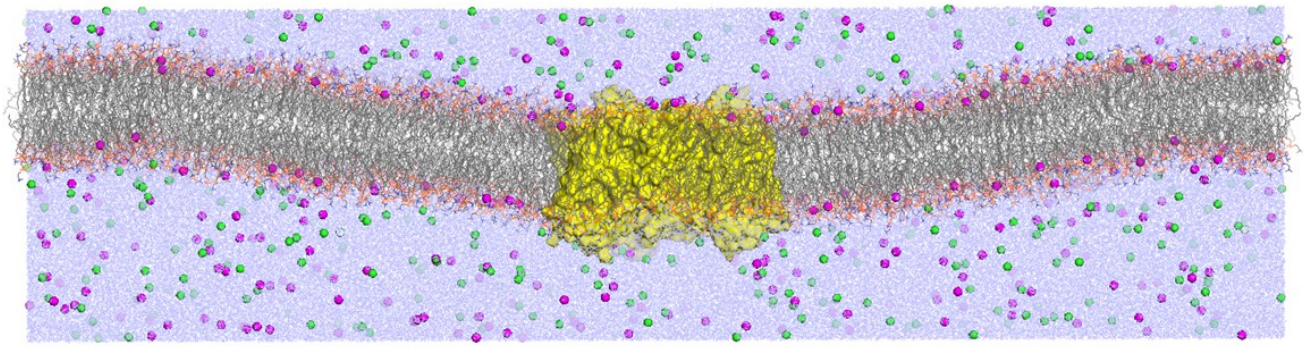
JGP The Journal of General Physiology

We thank The Journal of General Physiology for generously sponsoring the Roger Y. Tsien Memorial Lecture. The Journal and the Society have a long-standing collaborative relationship.



We would like to acknowledge and thank Christopher Davenport for his wonderful design of this year's Symposium logo.

Society of General Physiology
72nd Annual Symposium
Marine Biological Laboratory
Woods Hole, MA



Molecular physiology of the cell membrane: an integrative perspective from experiment and computation

Organizers

José D. Faraldo-Gómez (NIH) &
Janice L. Robertson (U Iowa)

Friends of Physiology Keynote Speaker

Chris Miller (Brandeis U/HHMI)

Website

http://www.sgpweb.org/2018symposium

SGP Council 2017

President, Mark Shapiro

President-Elect, Merritt Maduke

Past-President, Robert Dirksen

Secretary, Andrew Harris

Treasurer, Frank Horrigan

Councilors

Sudha Chakrapani

Henry Colecraft

John Lueck

Andrea Meredith

Gilbert Martinez

Riccardo Olcese

Brad Rothberg

2017 Symposium Organizers

Richard Kramer (University of California Berkeley)

Edwin Levitan (University of Pittsburgh)

SGP Membership

SGP was founded in 1946 "to promote and disseminate knowledge and interest in the subject of general physiology". Our international membership is made up of physiologists who work in academia, government, and industry. We have become known for promoting and nurturing research related to membrane transport and ion channels; membrane structure, regulation, and dynamics; and cellular contractility and molecular motors.

The major activity of the Society is its annual symposium at the Marine Biological Laboratory in Woods Hole, which members can attend at a discounted rate. SGP symposia cover the forefront of physiological research and are small enough to maximize discussion and interaction.

Membership in the Society is open to any individual actively interested in the field of general physiology. Categories of membership include regular, young investigator, and emeritus. The Membership year runs annually from January 1st to December 31st.

Save the Date!

The Society of General Physiologists

& The Journal of General Physiology

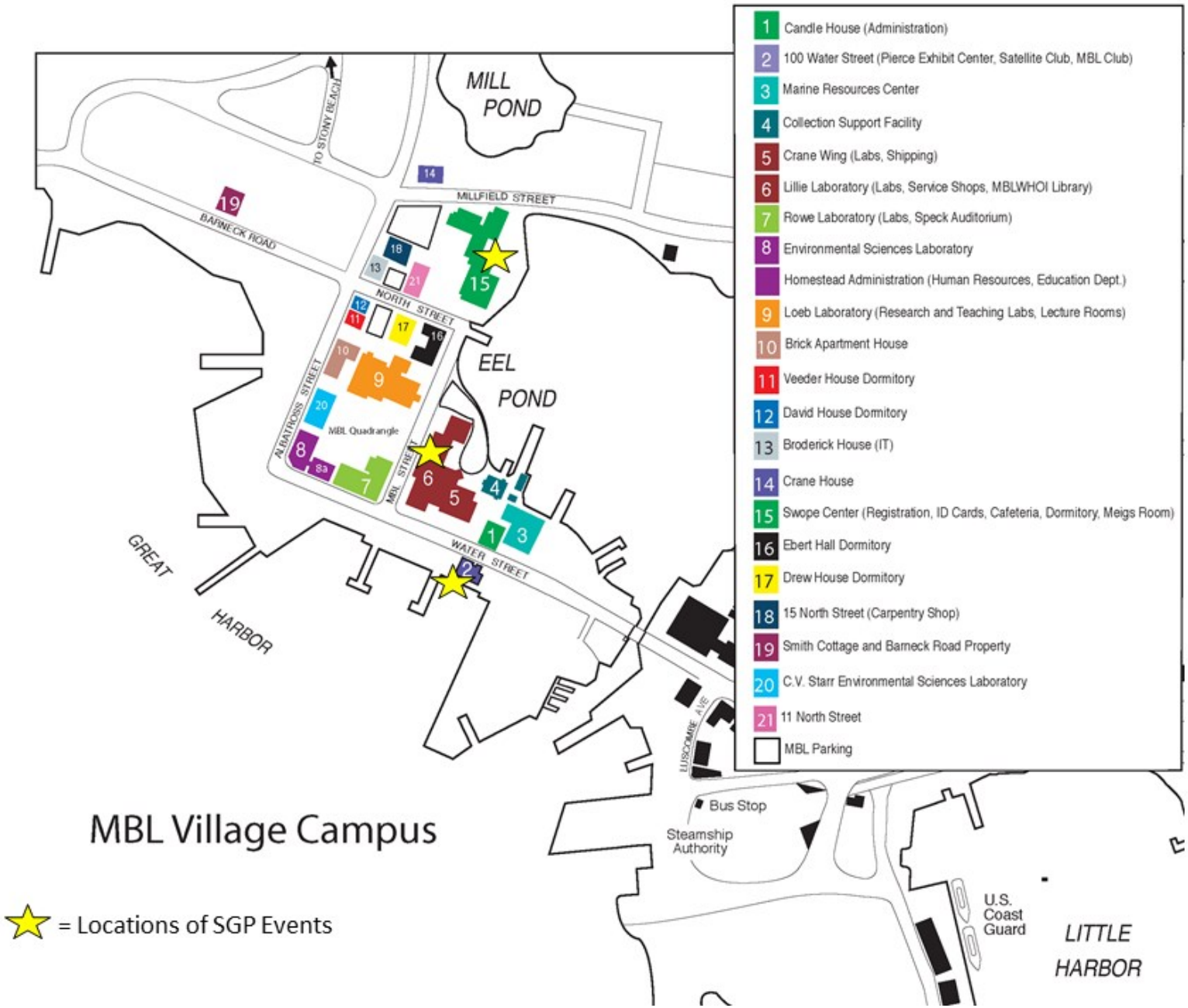
6th Annual Mixer

18 Feb 2018

San Francisco, CA

(Sunday of Biophysics Meeting)

Please join us and connect with fellow physiologists,
learn about SGP membership benefits,
& the upcoming 2018 SGP Symposium


MILL POND
TO STONY BEACH
MILLFIELD STREET
BARNECK ROAD
NORTH STREET
EEL POND
ALBATROSS STREET
MBL Quadrangle
MBL STREET
WATER STREET
GREAT HARBOR
LUSCOMBE AVE
Bus Stop
Steamship Authority
U.S. Coast Guard
LITTLE HARBOR
1 Candle House (Administration)
2 100 Water Street (Pierce Exhibit Center, Satellite Club, MBL Club)
3 Marine Resources Center
4 Collection Support Facility
5 Crane Wing (Labs, Shipping)
6 Lillie Laboratory (Labs, Service Shops, MBLWHOI Library)
7 Rowe Laboratory (Labs, Speck Auditorium)
8 Environmental Sciences Laboratory
Homestead Administration (Human Resources, Education Dept.)
9 Loeb Laboratory (Research and Teaching Labs, Lecture Rooms)
10 Brick Apartment House
11 Veeder House Dormitory
12 David House Dormitory
13 Broderick House (IT)
14 Crane House
15 Swope Center (Registration, ID Cards, Cafeteria, Dormitory, Meigs Room)
16 Ebert Hall Dormitory
17 Drew House Dormitory
18 15 North Street (Carpentry Shop)
19 Smith Cottage and Barneck Road Property
20 C.V. Starr Environmental Sciences Laboratory
21 11 North Street
MBL Parking
MBL Village Campus
= Locations of SGP Events

Important Meeting Site Information

Registration: Meeting registration takes place on the first floor of the Swope Center from 2:00 to 9:00 p.m. on Wednesday, September 6. Check-in is at the main desk in Swope Lobby (opposite the door) and your registration packets are at the first table on your left. If you arrive during the night, instructions are posted at Swope Center on how to contact MBL Security who will have your room key.

Lectures: Will be held in the Speck Auditorium, Rowe Building (on MBL Street). Coffee breaks will be set up in the foyer outside of the auditorium, with more mingling and seating space just outside. Timing is tight, so it's important to be on time for lectures and to return quickly from the 20-minute breaks.

Poster Instructions: Poster boards are located on the second floor of Swope Center. Posters may be up to 42" wide x 60" high (portrait-style). Thumbtacks are provided. Poster numbers and titles are listed in the abstracts section of this program. Posters will be presented in two sessions.

Poster Session A: Posters may be displayed after 8:00 a.m. on Thursday, and must come down by Friday noon.

Poster Session B: Posters may be displayed after 3:00 p.m. on Friday afternoon, and must come down by Saturday evening.

Mixers: All mixers will be held in the Meigs Room in the Swope Center.

Meals: Conference meals are included in your registration, even if you are staying offsite. All meals are served in Swope Dining Hall overlooking Eel Pond. There are three meals a day starting with Dinner on day of arrival and ending with Lunch on the day of departure. There will be breakfast and a box lunch on the last day. There are selections for those with dietary restrictions including gluten-free, vegetarian, vegan, and nut- or sugar-free desserts. *N.B. The dining hall will stop serving lunch at 1:30 p.m.*

MBL's Cards: Every participant is issued an MBL ID card (proximity card) at registration. This card, *to be kept on you at all times*, will provide 24-hour access to Swope, and your dormitory building. Many buildings, including the dormitories, have automatically locking doors. Although Swope will be open until 9 p.m., other buildings will always be locked and you will need your proximity card to gain access. If you should have any questions, both MBL and SGP personnel will be available to answer them during the course of the meeting.

Internet Access: MBL has wireless internet service throughout its campus. To use the system simply browse for wireless networks on any wireless enabled device and select "MBL-Guest". Open a web browser and you will be automatically redirected to a login page. Enter the login credentials: user name = mblguest, password = mblguest. In addition, there are 6 terminals from which you can check your email or do a web search in Swope Center. For other computer activity, the Computer Room is located in the MBL Library, Lillie 204.

Parking: If you drive to Woods Hole, remember that *parking is by permit only*. You must obtain a parking permit at the registration desk and park ONLY at the Bar Neck parking lot. Directions will be given to you at the registration desk. Do NOT park in any of the other MBL lots or on the street—ticketing and towing are considered a local recreation.

Luggage Storage: There is a carriage house outside Swope where luggage can be stored if you have arrived early or need to store your items beyond check out. The front desk staff will assist you in locating the building should you need help.

Library Usage: The MBL Library has a world-renowned collection, and visitors are welcome at all times. The Library is staffed Monday through Friday, 8:00 a.m. to 5:00 p.m.

Mail/Messages: Private phones are available in nearly all guest rooms. Messages can be left at 508-548-3705 24 hours a day and are available for pickup at the front desk of Swope Center (phones in rooms do not have voice mail). Packages and mail should be addressed to your name, *Society of General Physiologists, Marine Biological Laboratory, 7 MBL Street, Woods Hole, MA 02543-1015.*

71st Annual Meeting and Symposium of the Society of General Physiologists

Marine Biological Laboratory,

Woods Hole, Massachusetts

September 6–10, 2017

The Optical Revolution in Physiology: From Membrane to Brain

ORGANIZED BY

Richard Kramer (University of California Berkeley)

and Edwin Levitan (University of Pittsburgh)

SCHEDULE OF EVENTS

WEDNESDAY, SEPTEMBER 6

2:00 pm – 9:00 pm	**Registration and Room Assignments (Swope Center, 1st Floor)**
5:30 pm – 7:00 pm	**Dinner (Swope Center, Dining Room, 2nd Floor)**
7:30 pm – 8:00 pm	**Remarks (Speck Auditorium, Rowe Building)** Mark Shapiro, President, Society of General Physiologists Conference Organizers, Richard Kramer and Edwin Levitan
8:00 pm – 9:00 pm	**Friends of Physiology Keynote Lecture** **Eric Betzig (HHMI Janelia)** *Imaging Life at High Spatiotemporal Resolution*
9:00 pm – 11:00 pm	**Mixer (Meigs Room, Swope Center)** *Travel Awards Presented*

THURSDAY, SEPTEMBER 7

7:00 am – 8:30 am **Breakfast (Swope Center, Dining Room)**

8:30 am – 9:40 am **Session I: Seeing Single Molecules in Cells (Speck Auditorium)**

Biotium has generously supported this session as a **Silver Sponsor**.

Xiaowei Zhuang (Harvard)
New technology for super-resolution of single molecules in cells

Thomas Blanpied (University of Maryland)
High resolution visualization of dynamic protein organization in synapses

9:40 am–10:00 am **Break**

10:00 am–11:40 am **Session II: Imaging Cell Signaling**

3i Intelligent Imaging Innovations has generously supported this session as a **Silver Sponsor**.

Marie Burns (UC Davis)
In vivo AO-SLO/OCT imaging of microglia during the initiation and resolution of retinal degeneration

Jin Zhang (UCSD)
Illuminating the biochemical activity architecture of the cell

Robert Campbell (University of Alberta)
Development of a new generation of optogenetic tools for visualization and manipulating cell signaling

11:40 am–12:00 pm **Poster Blitz Preview A**

Abstract 8: *In vivo* multiphoton imaging of polymer dot-labeled vasculature in C57 mice **Ahmed Hassan**
Abstract 10: Red-to-green photoconversion of mCherry as a source of potential artifacts in multicolor labeling experiments **Yusheng Jiang**
Abstract 24: Opsin 3-mediated regulation of skin pigmentation **Lauren Olinski**
Abstract 34: Defining olfactory bulb functions via comparison of input and output **Douglas Storace**
Abstract 44: Wide-field optical mapping (WFOM) of large-scale neural activity and hemodynamics in the awake mouse cortex **Mohammed Shaik**

12:00 pm –1:30 pm **Lunch (Dining Room, Swope Center)**

1:30 pm – 3:00 pm **Free Time**

THURSDAY, SEPTEMBER 7—Continued

3:00 pm–5:00 pm **Poster Session A (Swope Center, 2nd Floor)**
Session A authors at posters for discussion with attendees

5:30 pm–6:45 pm **Dinner (Swope Center, Dining Room)**

7:00 pm–8:45 pm **Session III: Imaging membrane trafficking and secretion**

Chroma has generously supported this session as a **Silver Sponsor**.

Edwin Levitan (University of Pittsburgh)
Neuronal organelle delivery, capture, and multitasking

Dalibor Sames (Columbia University)
Chemists to a chemical synapse: Imaging synaptic release with fluorescent false neurotransmitters

Lin Tian (UC Davis)
Genetically encoded sensors for monitoring release of biogenic amines and neuromodulation

9:00 pm–11:00 pm **Mixer (Meigs Room, Swope Center)**

FRIDAY, SEPTEMBER 8

7:00 am–8:30 am **Breakfast (Dining Room, Swope Center)**

8:30 am–9:40 am **Session IV: Optical Sensors of Electrical Activity**

Nikon has generously supported this session as a **Silver Sponsor**.

Evan Miller (UC Berkeley)
Electrophysiology: unplugged. New fluorescent sensors to image voltage

Adam Cohen (Harvard University)
All-optical electrophysiology in rodent CNS

9:40 am–10:00 am **Break**

10:00 am–11:10 am **Session IV *Continued***

Loren Looger (HHMI Janelia)
Engineering proteins to light up brain activity

Joshua Levitz (Cornell University)
*Optical dissection of G protein-coupled receptor*s

FRIDAY, SEPTEMBER 8—Continued

11:10 am–12:10 pm **Short Talks I (10 min each, 5 min questions)**

Masayuki Yazawa (Columbia University)
Developing optogenetic tools for tethering mitocondria and endoplasmic reticulum in neurons

Rosana Molina (Montana State University)
Finding two-photon brighter green fluorescent proteins in obscure, blue-shifted variants

Robert G. Stewart (UC Davis)
Imaging endogenous neuronal Kv2 potassium channel gating with a fluorescent tarantula toxin

Chase Carver (University of Texas HSC San Antonio)
Super resolution STORM quantitative analysis of nanodomain protein interactions in neurons

12:10 pm–1:30 pm **Lunch (Swope Center, Dining Room)**

1:45 pm–5:00 pm **FREE AFTERNOON: Harbor Cruise Option 2:00 pm–3:30 pm**
No charge. Sign-up sheet at registration table. Limited space.

5:00 pm–6:30 pm **Dinner (Dining Room, Swope Center)**

6:30 pm–7:20 pm **Roger Y. Tsien Memorial Lecture**
Atsushi Miyawaki (RIKEN)
Cruising inside cells: New fluorescent probes and new perspectives in bioscience

The Journal of General Physiology has generously supported this lecture.

7:20 pm–7:30 pm **Break**

7:30 pm–9:15 pm **Session V: Optical Studies of Synapses**

Donald Arnold (USC)
Visualizing and ablating synapses in vivo using novel recombinant probes

Ryohei Yasuda (Max-Planck Inst., Florida)
Imaging biochemical events underlying long-term synaptic plasticity

Richard Kramer (UC Berkeley)
Seeing the flip side of LTP through optogenetic manipulation of $GABA_A$ receptors

FRIDAY, SEPTEMBER 8—Continued

9:10 pm–9:30 pm **Poster Blitz Preview B**

Abstract 1: All-optical electrophysiology in behaving mice with enhanced near infrared voltage sensors **Yoav Adam**
Abstract 11: Recording neural activity in freely behaving animals with 3D tracking two-photon microscopy **Doycho Karaguozov**
Abstract 25: Two-photon imaging of neuronal activity in freely-behaving mice using a miniature fiber-coupled microscope with active axial focusing **Baris Ozbay**
Abstract 29: Source-localised multifocal two-photon microscopy for high speed functional imaging **Peter Quicke**
Abstract 39: Co-translational regulation of potassium and sodium channel expression mediated by mRNA interaction **Catherine Eichel**

9:30 pm–11:00 pm **Mixer (Meigs Room, Swope Center)**

SATURDAY, SEPTEMBER 9

7:00 am–8:30 am **Breakfast (Swope Center, Dining Room)**

8:45 am–10:30 am **Session VI: Whole Brain Imaging (Speck Auditorium)**

Leica Microsystems has generously supported this session as a **Gold Sponsor.**

Elizabeth Hillman (Columbia University)
New developments in SCAPE microscopy for high-speed 3D neuroimaging

Na Ji (HHMI Janelia)
Probing neural circuits with shaped light

10:30 am–10:50 am **Break**

10:50 am–11:25 am **Session VI *Continued***

Ed Boyden (MIT)
Optical tools for analyzing and repairing complex biological systems

Viviana Gradinaru (Caltech)
New approaches for cell-specific targeting of optogenetic tools

SATURDAY, SEPTEMBER 9—Continued

11:25 am–11:55 pm **Short Talks II**

Kotaro Kimura (Osaka University)
Calcium dynamics regulating the timing of decision-making in C. elegans

John M. Ferrante (Q-State Biosciences)
Phenotypic assays of synaptic transmission using all-optical electrophysiology

12:00 pm – 1:30 pm **Lunch (Dining Room, Swope Center)**

2:00 pm–2:30 pm **SGP Annual Meeting—All members welcome! (Speck Audit.)**

3:00 pm–5:00 pm **Poster Session B (Swope Center, 2nd Floor)**
Session B authors at posters for discussion with attendees

5:00 pm–7:00 pm **Lobster Dinner/Clambake (Dining Room, Swope Center)**

7:00 pm–8:45 pm **Session VII: Imaging and Manipulating Brain Activity *In Vivo***

Yves De Koninck (Laval University)
Imaging and manipulating pain in vivo

Spencer Smith (UNC Chapel Hill)
Panoramic views of the brain: next-generation multiphoton imaging

Daniel Dombeck (Northwestern University)
Exploring the brain's navigation system with high-resolution imaging and virtual reality

8:45 pm – 9:00 pm **Discussion: The Future of Optical Physiology**

9:00 pm – 11:00 pm **Mixer & Awards Ceremony (Meigs Room, Swope Center)**
Poster Awards

SUNDAY, SEPTEMBER 10

7:00 am–8:30 am **Breakfast (Dining Room, Swope Center)**

Pick up box lunches

10:00 am **Check-out deadline**

ABSTRACTS OF PAPERS AT THE SEVENTY-FIRST

ANNUAL MEETING OF THE SOCIETY

OF GENERAL PHYSIOLOGISTS

The Optical Revolution in Physiology: From Membrane to Brain

Marine Biological Laboratory

Woods Hole, Massachusetts

6–10 September 2017

Organized by

RICHARD KRAMER and EDWIN LEVITAN

1. All-Optical Electrophysiology in Behaving Mice With Enhanced Near Infrared Voltage Sensors. YOAV ADAM,[1] SHAN LOU,[1] and ADAM COHEN,[1,2] *[1]Department of Chemistry and Chemical Biology, Harvard University, Cambridge, MA; [2]Howard Hughes Medical Institute*

The ability to record and perturb optically from multiple neurons in the intact brain at single spike resolution will open new frontiers in neuroscience research. We combined protein engineering, novel microscopy techniques and targeted gene expression to develop a set of tools for "all-optical electrophysiology" in the brain of awake, behaving mice. We first engineered an improved near infrared voltage sensor called QuasAr3, which allowed high-fidelity recording of somatic and dendritic action potentials and sub-threshold events in acute brain slices. We then developed a Cre-dependent transgenic mouse line expressing both QuasAr3 and the optically orthogonal channelrhodopsin CheRiff and used it for all-optical classification of neuronal subtypes based on their firing patterns. We also developed an indicator called paQuasAr3, a photo activated variant that showed brighter fluorescence and improved signal-to-noise ratio (SNR) upon activation with blue light. Sparse expression of paQuasAr3 reported spontaneous spiking activity at high SNR in the hippocampus (CA1) and olfactory bulb of anesthetized mice.

We next sought to record from and manipulate multiple cells simultaneously. We developed soma-restricted versions of QuasAr3, paQuasAr3, and CheRiff, which enabled dense expression by significantly reducing the neuropil background. To further improve the imaging contrast, reduce the overall laser power, and allow precise spatial control, we patterned the stimulation and imaging lasers with a digital micromirror device. The combination of dense expression and patterned illumination allowed us to record from multiple cells, simultaneously in the hippocampal CA1 region of behaving mice and to detect running dependent supra- and subthreshold voltage dynamics. This set of tools opens the possibility to decipher neuronal circuits at high spatiotemporal resolution and unprecedented throughput.

2. Neuronal Responses of Ca^{2+} Transients in Interstitial Cells of Cajal in the Small Bowel. SALAH A. BAKER, BERNARD T. DRUMM and KENTON M. SANDERS, *Department of Physiology and Cell Biology, University of Nevada School of Medicine, Reno, NV 89557*

Introduction: Interstitial cells of Cajal (ICC) regulate of smooth muscle excitability and motility in the gastrointestinal (GI) tract. ICC in the deep muscular plexus (ICC-DMP) of the small intestine are closely associated with varicosities of enteric motor neurons and are suggested to transduce neural responses. ICC-DMP fire spontaneous Ca^{2+} transients linked to activation of Ca^{2+}-activated Cl^- channels, but modulation of Ca^{2+} transients by enteric neural inputs has not been described in detail.

Aim: Measure Ca^{2+} transients in ICC-DMP in situ that are elicited or suppressed by excitatory and inhibitory motor neural inputs.

Methods: We used the Cre-loxP technique to express GCaMP3 in combination with confocal imaging. Electrical field stimulation (EFS) was used to activate enteric motor neurons.

Results: EFS elicited inhibitory and excitatory effects on Ca^{2+} transients. Inhibitory nerve stimulation suppressed Ca^{2+} transients in ICC-DMP (from 66 transients/min to 2.9 transients/min). At termination of EFS, there was a period (~5–12s) of greatly enhanced Ca^{2+} transient activity (increased to 125 transients/min). Inhibitory effects on Ca^{2+} transients were mediated mainly by nitrergic neurotransmission, as the NO synthase inhibitor L-NNA blocked inhibitory effects. Prior to EFS, L-NNA increased spontaneous Ca^{2+} transients, and the NO donor, NONATE, inhibited Ca^{2+} transients. Furthermore, the soluble guanylate cyclase activator, BAY 58-2667, also decreased Ca^{2+} transients in ICC, indicating the inhibitory effects of nitrergic neurotransmission in ICC-DMP are mediated by the GC-cGMP pathway. Excitatory Ca^{2+} responses in ICC were reduced by atropine and the neurokinin 1 receptor (NK1) antagonist RP-67580 but not by an NK2 antagonist, MEN-10376. Exogenous ACh and substance P increased Ca^{2+} transients while atropine and RP-67580 decreased Ca^{2+} transients in ICC-DMP.

Conclusions: Excitatory and inhibitory neurotransmitters modulate Ca^{2+} transients in ICC-DMP. These observations demonstrate direct innervation and postjunctional responsiveness of ICC-DMP in intestinal muscles. Ca^{2+} transients in ICC-DMP activate inward currents that can regulate the excitability of the small intestine provides a signaling mechanism responsible for transduction of enteric neuronal input.

3. Super-Resolution STORM Quantitative Analysis of Nanodomain Protein Interactions in Neurons. CHASE M. CARVER and MARK S. SHAPIRO, *Department of Cellular and Integrative Physiology, University of Texas Health Science Center San Antonio, TX*

Fidelity of neuronal signaling requires organization of signaling molecules into highly proximal macromolecular complexes. The intrinsic diffraction limit of light makes visualization of individual signaling complexes difficult. However, using multi-color super-resolution stochastic optical reconstruction microscopy (STORM), we observed intimate association of individual molecules within signaling complexes containing ion channels (M-type K^+, L-type Ca^{2+}, or TRPV1 channels) and G protein–coupled receptors coupled by A-kinase–anchoring protein (AKAP)79/150 as a scaffold. We identified novel interplay of complexes containing diverse channel types in sensory neurons, dependent on AKAP79/150. Electrophysiological studies confirmed

functional coupling. We also determined the capability of STORM to image physical interactions spanning the ER and plasma membrane.

Beyond nearest-neighbor localization analysis, STORM-derived localizations can be algorithmically characterized by proximity and density to signify interactions of the labeled proteins. We developed an in-house density-based clustering (DBSCAN-like) algorithm to objectively categorize labeled protein interactions with stringent parameters, rather than selecting clusters by eye. We used population statistics of STORM cluster data derived from a large sample of cells to analyze cluster size, cluster localization number, and overall percentage of each possible cluster outcome derived from two- or three-color labeling. Therefore, we used the total population distributions of clusters categorized by type and size to probabilistically determine various states of channel interactions.

Our findings illustrate the novel role of AKAP150 as a molecular coupler of different channels with unique functions within single nanodomains in tuning the physiological response of sensory neurons. The cluster analysis demonstrates macromolecular complexes in competition for AKAP150, evident from the limited existence of unpaired AKAP150 in sensory neurons. We demonstrate a novel methodology for quantitatively understanding spatial interactions under STORM. Such methods can visualize dynamic states of channel–complex interactions that mirror structural and functional changes in neuronal signaling.

4. Optical Imaging and Labeling of Individual Biomolecules in Dense Clusters. MINGJIE DAI,[1,2,3] RALF JUNGMANN,[1,2,4] NINNING LIU,[1,2] and PENG YIN,[1,2] *[1]Wyss Institute for Biologically Inspired Engineering, Harvard University, Boston, Massachusetts 02115; [2]Department of Systems Biology, Harvard Medical School, Boston, Massachusetts 02115; [3]Biophysics Program, Harvard University, Boston, Massachusetts 02115; [4]Present address: Max Planck Institute of Biochemistry and LMU, Munich 82152, Germany*

Recent advances in fluorescence super-resolution microscopy have allowed subcellular features and synthetic nanostructures down to ~15 nm in size to be imaged. However, direct optical observation of individual molecular targets (~5 nm) in a densely packed biomolecular cluster remains a challenge. Here, we show that such discrete molecular imaging is possible using DNA-PAINT (points accumulation for imaging in nanoscale topography)—a super-resolution fluorescence microscopy technique that exploits programmable transient oligonucleotide hybridization—on synthetic DNA nanostructures. We examined the effects of high photon count, high blinking statistics, and appropriate blinking duty cycle on imaging quality and developed a software-based drift correction method that achieves <1-nm residual drift (r.m.s.) over hours. This allowed us to image a densely packed triangular lattice pattern with ~5-nm point-to-point distance, and analyze DNA origami structural offset with angstrom-level precision (2 Å) from single-molecule studies. By combining the approach with multiplexed Exchange-PAINT imaging, we further demonstrated an optical nano-display with 5 × 5–nm pixel size and three distinct colors and with <1-nm cross-channel registration accuracy. Combined with photo-activated cross-linker, we further demonstrated selective labeling on single molecule targets with custom patterns and subdiffraction (~30 nm) resolution. These methods open up possibilities for direct and quantitative optical observation and perturbation of individual biomolecular features in crowded environments.

5. Increase in Spontaneous Neuronal Activity Occurs During a Stage of Vascular Expansion in the Rat Auditory Brainstem During Postnatal Development. MARIANO DI GUILMI,[1,2] CHEIKH BABOU,[1] and ADRIÁN RODRÍGUEZ-CONTRERAS,[1] *[1]The City University of New York, City College, Biology Department; [2]Laboratorio de Fisiología y Genética de Audición, INGEBI, Argentina.*

Neurovascular coupling reflects the metabolic and signaling relationship between vascular endothelial and neural cells. However, little is known about the developmental origins of neurovascular coupling. Before the onset of hearing around postnatal day 12 (P12), auditory brainstem neurons in the medial nucleus of the trapezoid body (MNTB) of rats fire spontaneous bursts of action potentials (Tritsch et al. 2010. *Nature Neurosci.* 13:1050–1052), cell proliferation increases, and astrocytes differentiate (Saliu et al. 2104. *J. Comp. Neurol.* 522:971–985), suggesting that developmental changes may occur in neurovascular coupling. In this study, developmental changes in vascular network structure and neuronal ensemble activity were examined in the MNTB of rat pups. Development of the vascular network was examined with isolectin-B4 and EdU histochemistry in 80-µm thick horizontal brainstem sections (n = 19 pups). In separate experiments, ensemble neuronal activity was measured with 16-channel multi-electrode arrays placed in the MNTB of isoflurane or ketamine anesthetized pups (n = 30 pups). Although vascular volume (in μm^3) was constant between P0 and P5 (15,879 ± 1,147 and 16,654 ± 906, respectively), a marked increase was detected at P10 (30,915 ± 2,502; Student's t test, $P < 0.00001$). EdU labeling confirmed cell proliferation of vascular and perivascular cells at P6 and P7. Electrophysiology recordings showed that multi-unit activity could be detected as early as P3, but with a noticeable increase at P6. Altogether, this data shows coordinated changes in vascular volume and neuronal activity during the prehearing stage of rat postnatal development.

6. Fluorescent False Neurotransmitter 270: New Tracer for Examining Norepinephrine Synaptic Function In Vivo. MATTHEW DUNN, DAVID SULZER, and DALIBOR SAMES, *Departments of Chemistry, Neurology, Pharmacology, and Psychiatry, Columbia University, New York, NY*

Norepinephrine is a monoamine neurotransmitter with a wide repertoire of physiological roles in the peripheral and central nervous systems, including regulation of stress responses, arousal, and cognition. Despite major strides in understanding the noradrenergic system, limited experimental means for examining neurotransmitter release at discrete presynaptic boutons have prevented studying the functional properties of individual noradrenergic synapses in the brain. To address this need, we expanded the fluorescent false neurotransmitters (FFNs) platform developed in our laboratories to this neurotransmitter system. We identified FFN270 as a novel fluorescent and pH-sensitive substrate of both the norepinephrine transporter and the vesicular monoamine transporter. As such, FFN270 traces norepinephrine transmission using the native protein machinery already specific to these neurons, providing a novel approach to optically study the regulation of neurotransmitter uptake, vesicular loading, and synaptic vesicle content release of norepinephrine presynaptic synapses. Using FFN270, we were able to access important physiological parameters, such as synaptic release probability of individual terminals, changes in vesicular pH, and neurotransmitter release events even in sparsely innervated cortical areas. Through specific optogenetic activation of the norepinephrine system, we observed heterogeneous release properties from individual norepinephrine release sites in the barrel cortex, identifying distinct low and high releasing populations. We also report the first measurement of monoamine neurotransmitter release with single synapse resolution in the living mouse, using both optogenetics and pharmacological activation with amphetamine. In conclusion, FFN270 provides a readout of synaptic activity within the norepinephrine system that is currently not possible with other tools in the field and can prove invaluable in studying synaptic function and regulation.

7. Phenotypic Assays of Synaptic Transmission Using All-Optical Electrophysiology. JOHN M. FERRANTE, FELIPE GERHARD, HANSINI UPADHYAY, GABRIEL BORJA, HIMALI SHROFF, STEVEN NAGLE, LUIS A. WILLIAMS, OWEN MCMANUS, KIT WERLEY, and GRAHAM T. DEMPSEY, *Q-State Biosciences Inc., Cambridge, MA, 02139*

A multitude of studies have implicated synaptic dysfunction in neurological diseases, including forms of epilepsy, Alzheimer's disease, Parkinson's disease, autism spectrum disorder (ASD), schizophrenia, and affective disorders. However, conventional methods of measuring synaptic activity are limiting: manual patch clamp has exquisite information but throughput is very low, while fluorescent Ca^{++} assays are high-throughput but cannot resolve subthreshold postsynaptic potentials (PSPs). To address these shortcomings, we developed synaptic assays based on Optopatch, which is comprised of an engineered channelrhodopsin, CheRiff, to stimulate cells with blue light, and an engineered fluorescent protein, QuasAr, to record voltage with red light. Using Cre-dependent constructs, we express CheRiff and QuasAr in mutually exclusive subsets of cells, allowing us to stimulate pre-synaptic cells and record PSPs from their postsynaptic partners. Our imaging system allows the simultaneous recordings of PSPs from ~20–50 individual neurons within a 0.5 × 4–mm FOV with 1-ms temporal resolution. Using combinations of synaptic blockers, we pharmacologically isolate GABAR/NMDAR/AMPAR signals and extract excitatory and inhibitory PSP properties, including amplitude, rise time, decay time, width, and area from tens of thousands of cells in a single day. In addition to high-throughput assays of PSPs, the platform can interrogate other critical aspects of synaptic function. We will discuss preliminary results, including (1) interrogation of network connectivity and microcircuits by targeted stimulation of individual cells with a digital mirror device (DMD); (2) resolving synaptic behaviors between different neuronal types using selective promoters (e.g., *dlx1*); (3) studying long- and short-term potentiation (LTP and STP); and (4) quantifying NMDAR and AMPAR PSPs in human induced pluripotent stem (hIPS) cell–derived excitatory neurons. The high content and scalability of the Optopatch synaptic assay, in addition to its applicability to human cultures, make it a powerful tool in resolving synaptic phenotypic responses in neurological diseases and screening candidate therapeutics.

8. In Vivo Multiphoton Imaging of Polymer Dot-Labeled Vasculature in C57 Mice. AHMED M. HASSAN,[1] JEREMY W. JARRETT,[1] DAVID R. MILLER,[1] YEN-LIANG LIU,[1] HSIN-CHIH YEH,[1] and ANDREW K. DUNN,[1] *[1]Department of Biomedical Engineering, The University of Texas at Austin, Austin, TX*

In vivo bioimaging has emerged as the experimental foundation of many labs spanning a diverse range of research disciplines. Of the various approaches, multiphoton microscopy (MPM) is particularly well suited for deep, in vivo imaging due to its non-invasiveness and ability to maintain high spatial resolution at depth through a nonlinear dependence on excitation intensity. MPM demands bright, photostable contrast agents to overcome the strong absorption and scattering events that occur in biological tissue. Polymer dots (pdots) emerge as a highly emissive class of probes with promising characteristics and immense potential for the next

generation of bioimaging. Here, we measure the two- and three-photon power dependence of three pdot variants (CNPPV, PFBT, and PFPV) and demonstrate deep neuroimaging of pdot-labeled mice. We find that all three pdots demonstrate a 2P power dependence across the titanium:sapphire tuning range and a 3P dependence at longer wavelengths using an optical parametric amplifier (OPA). In addition, we show that pdots' action cross sections are one to two orders of magnitude brighter than quantum dots and three to five orders of magnitude larger than conventional fluorescent dyes and proteins in the near-infrared regime. Their extraordinary brightness enables 2P imaging of vasculature in mice at a depth of 850 µm with excellent SBR. We also find that pdots' broad absorption confers compatibility with a high-power ytterbium-fiber (yb-fiber) laser and a high pulse energy OPA. Taking advantage of the unique properties of these laser systems, the intrinsic reduction of scattering of longer wavelengths, and the suppression of out-of-focus fluorescence introduced by higher order non-linear excitation, we extend imaging depth ($z_{\text{yb-fiber}} = 900$ µm, $z_{\text{OPA}} = 1300$ µm) and improve SBR substantially. With these brighter probes, researchers will be able to perform in vivo imaging with improved clarity and depth, enabling critical insights into fundamental biological problems.

9. High-Throughput FRET Assays for cAMP Using the Epac1-Based Biosensor H188 Enable GPCR Agonist Drug Discovery. GEORGE G. HOLZ , COLIN A. LEECH, and OLEG G. CHEPURNY, *State University of New York Upstate Medical University, Syracuse, NY*

High-throughput screening of small molecule libraries is one means by which to identify new pharmacological agents that activate G protein–coupled receptors (GPCRs) to stimulate cAMP production so that ligands with beneficial medicinal properties are discoverable. Here, we report a new microplate reader assay that allows high-throughput detection of cAMP with fast temporal resolution and wide dynamic range for cells treated with GPCR agonists. The assay uses clonal HEK293-H188 cells that express the genetically encoded biosensor H188 in which the cAMP-binding protein Epac1 is flanked by mTurquoise2Δ and ^{cp173}Venus-Venus FRET donor/acceptor fluorophores. Analysis of HEK293-H188 cell monolayers demonstrates that this assay is suitable to monitor the increase or decrease of cAMP levels that results when GLP-1, GIP, Glucagon, Adenosine-2B, TGR5, and NPY2R receptors are stimulated by their respective agonists. Accurate dose–response analysis is made possible by the nearly 10-fold greater ΔFRET that H188 exhibits when compared with the first-generation biosensor Epac1-camps. By adapting this assay to studies of cyclic nucleotide analog action in living cells, we also define the properties of Epac1 activators and inhibitors with great precision. Thus, this microplate reader FRET assay enables GPCR drug discovery, while providing a new platform with which to advance cyclic nucleotide research.

10. Red-to-Green Photoconversion of mCherry as a Source of Potential Artifacts in Multicolor Labeling Experiments. JASON Y. JIANG, JEFFREY L. FALCONE, SILVANA CURCI, and ALDEBARAN M. HOFER, *Dept. of Surgery, VA Boston Healthcare System, Brigham and Women's Hospital and Harvard Medical School*

The monomeric red fluorescent protein mCherry from the soft coral *Discosoma* is considered among the most stable and rapidly maturing of the red fluorescent proteins (RFPs), accounting for its widespread popularity as a protein tag and constituent of genetically encoded sensors. We observed significant "greening" of mCherry and several other related RFPs upon illumination of the red fluorescence using power settings that are within normal limits for many types of fluorescence microscopy experiments (confocal and epifluorescence). Red-to-green behavior was observed when mCherry was expressed either alone or when fused to protein partners in several types of living cells, in fixed cells, and in droplets of purified protein over a wide range of protein concentrations. Although the green species that emerged after photobleaching was only about 10% as bright as mCherry, and dim compared to EGFP, we provide examples of how this characteristic can lead to significant artifacts during co-localization experiments employing green fluorescent proteins or when combined with genetically encoded calcium and cAMP sensors. Photoconverted mCherry thus has the potential to be confused with green FPs under many types of experimental conditions, for example, when GFP is used to report endogenous protein levels (as in transgenic animals or CRISPR knock-ins), or when combined with other green FPs that are not necessarily as bright as GFP. Our observations do not detract from the utility of mCherry, which remains one of the "best-behaved" RFPs available, but do highlight the importance of appropriate controls to assess the impact of red-to-green photoconversion in such situations.

11. Recording Neural Activity in Freely Behaving Animals With 3D Tracking Two-Photon Microscopy. DOYCHO KARAGYOZOV,[1] MIRNA MIHOVILOVIC SKANATA,[1] AMANDA LESAR,[1] and MARC GERSHOW,[1,2,3] *New York University, Department of Physics[1], Center for Neural Science[2], Neuroscience Institute[3]*

The *Drosophila* larva, a genetic model organism with a transparent cuticle and compact brain, is a nearly ideal substrate for optical neurophysiology. Optical recording of neural activity from behaving larvae has proved elusive due to rapid three-dimensional brain movements generated by peristaltic crawling. Recent developments in light sheet and extended depth of field

microscopy show promise in imaging moving subjects, but these techniques can be limited by scattering in tissue, and the excitation light presents an unwanted visual stimulus as well as an obstacle to simultaneous optogenetic manipulation. Here, we present a two-photon microscope capable of high bandwidth fluorescent recordings in three-dimensionally moving neurons without motion artifacts. Using conventional galvonometric mirrors and a resonant ultrasonic lens, we move the microscope's focal spot rapidly in a cylinder about the center of a single targeted neuron. We count the photons emitted from each point on the scan, use these to form an updated estimate of the neuron's location, and execute the next scan around the updated center. The algorithm is implemented in FPGA hardware, and the feedback loop closes in 360 microseconds. A secondary feedback moves the microscope stage to keep the neuron centered in the field of view. Because the focal spot never leaves the neuron, we achieve a continuous recording of fluorescence with a bandwidth exceeding 1 kHz.

To demonstrate the utility of the microscope, we recorded from individual aCC motor neurons in the ventral nerve cord and correlated their activities with the larva's peristaltic motion, visualized simultaneously on an infrared video camera. We then tracked visual interneurons and recorded their responses to blue light stimuli. Finally, we simultaneously tracked and recorded from two adjacent neurons. To our knowledge, this is the first two-photon recording of neural activity in which the brain was not rigidly coupled to the microscope.

12. Optogenetic Cre/loxP recombination system with high efficiency. FUUN KAWANO[1] and MASAYUKI YAZAWA,[1,2] [1]*Department of Rehabilitation and Regenerative Medicine, Columbia University, New York, NY 10032;* [2]*Department of Pharmacology, Columbia University, New York, NY 10032*

Site-specific DNA recombination systems represented by Cre/*loxP* are a powerful tool for modifying transgenes in chromosomes of living organisms. A variety of conditionally inducible Cre/*loxP* recombination systems have been developed in combination with cell-, tissue-, or developmental stage–specific promoters and/or chemically inducible systems. However, such conventional approaches have relatively poor spatiotemporal resolution. Recently emerged optogenetic approaches are superior in terms of high tunability, rapid tissue penetration, and non-invasiveness in addition to high spatial and temporal confinement. However, powerful and useful Cre/*loxP* recombination techniques based on optogenetic systems remain elusive. Here, we describe a genetically encoded photoactivatable Cre recombinase (named PA-Cre) with high efficiency to implement DNA recombination with light in living systems (Kawano et al. 2016. *Nat. Chem. Biol.* 12:1059–1064). PA-Cre is based on reassembling of split Cre fragments driven by heterodimerization of nMag and pMag that are photoswitches recently engineered from a blue light-absorbing fungal photoreceptor Vivid (Kawano et al. 2015. *Nat. Commun.* 6:6256). We fused nMag and pMag with CreN59 (residues 19–59) and CreC60 (residues 60–343) of split Cre fragments, respectively. We optimized them in terms of linker amino acid sequences and configurations on the basis of structural information of Cre and the Vivid dimer in the light state. As a result of the step-by-step improvements, we obtained PA-Cre that yields a substantially efficient DNA recombination (up to 320-fold induction) with high spatiotemporal precision upon blue light illumination in vitro and in vivo. Additionally, by taking advantage of nMag and pMag having slow photocycle kinetics ($t_{1/2}$ = 1.8 h), we demonstrated that PA-Cre enables DNA recombination in vitro and in vivo even through illumination for only a short period of time (~30 s). This robust technique will facilitate optogenetic genome engineering in living systems.

13. Adaptive Optics Confocal Microscopy for Voltage Imaging in Tissue and In Vivo. SIMON KHEIFETS, YOAV ADAM, DAAN BRINKS, and ADAM COHEN

Functional imaging using genetically encoded voltage indicators promises to enable new insights into neural dynamics. Potential applications include high-throughput multi-cell intracellular recording, as well as measurement of the propagation and compartmentalization of voltage within a neuron. Such measurements are hindered by the relatively low signal-to-noise ratio (SNR) currently achieved when imaging at millisecond time scales. Voltage imaging in intact mammalian brain tissue faces a unique combination of factors that negatively affect SNR. Besides the relatively low brightness and/or sensitivity of existing reporters, other confounding factors include the sparse, intertwined geometry of cell membranes, the high temporal bandwidth requirements, and the scattering, aberration and autofluorescence of mammalian brain tissue. The techniques that have been successful for calcium imaging are not optimal for voltage imaging. We have been developing microscopy techniques that optimize the SNR for voltage imaging in tissue. These techniques include aberration-corrected confocal microscopy for spatially resolved single-cell recordings, as well as holographic illumination for wide-field multi-cell recording. Preliminary results demonstrate robust optical recording of action potentials and subthreshold events in acute slice and in vivo.

14. Calcium Dynamics Regulating the Timing of Decision Making in *C. elegans*. KOTARO KIMURA, *Osaka University*

Brains process sensory information to generate various kinds of physiological responses with different timings

(i.e., with different latencies to respond; Buhusi and Meck. 2005. *Nat. Rev. Neurosci.* 6:755–765). In decision making, for example, animals choose one from multiple behavioral options based on environmental sensory information, where a temporal delay is associated with the certainty of sensory information (Gold and Shadlen. 2007. *Annu. Rev. Neurosci.* 20:535–574). The neural mechanism of timing, however, is largely unclear. We report here the cellular and molecular mechanisms underlying the timing of decision making during olfactory navigation in *Caenorhabditis elegans*. We found that, based on subtle changes in concentrations of the repulsive odor, the animals appear to choose the appropriate migratory direction from multiple trials as a form of behavioral decision-making. By analyzing neural responses in freely behaving animals under virtual odor gradients with calcium imaging, optogenetics, mathematical modeling, and genetics, we found that odor concentration information is temporally integrated for a decision by a gradual increase in intracellular calcium concentration ($[Ca^{2+}]_i$), which occurs via L-type voltage-gated calcium channels (VGCC) in a pair of AWB olfactory neurons. In contrast, for a reflex-like behavioral response, $[Ca^{2+}]_i$ rapidly increases via multiple types of calcium channels in a pair of ASH nociceptive neurons. Our data suggest that the timing of neuronal responses can be determined by cell type–dependent involvement of calcium channels and that a single-cell temporal integrator with L-type VGCCs, such as the AWB neuron, may be the evolutionarily conserved neural basis for decision-making.

15. Characteristics of the b2 Subunit of the Calcium-Activated K^+ Channel in Skate Electroreceptors.

BENJAMIN L. KING,[1] PETER KAO,[2] LING FANG SHI,[2] and WILLIAM T. CLUSIN,[2] *University of Maine[1] and Stanford University Medical School[2]*

Our comparative studies seek to understand the structure and function of ion channels through the study of cartilaginous fish electroreceptors that can detect voltage gradients as low as 5 nanovolts per centimeter. Existence of calcium activated K^+ channels in excitable electroreceptor cells of the skate, *L .erinacea*, was first proposed in 1974. In humans, the large conductance (B_K) channel is comprised of four α-subunits, encoded by *KCNMA1*, and modulatory β-subunits, encoded by *KCNMB1*, *KCNMB2*, *KCNMB3*, and *KCNMB4*. We recently cloned the skate *KCNMA1* gene using purified mRNA from homogenized isolated electroreceptors (King et al. 2016. *Gene.* 578:63–73). The skate *KCNMB4* subunit was also partially cloned in that study. Recently, Bellono et al. (2017. *Nature.* 543:391–396) performed RNA sequencing (RNA-Seq) on purified mRNA from skate electroreceptors and found several ion channels including *KCNMA1*. Because the β-subunits play an important role in modulating B_K channel activity, we searched the RNA-Seq data for β-subunits and identified skate *KCNMB2*. The transcript encodes a 279-aa protein that contained 51 additional amino acids at the N terminus compared with human *KCNMB2*. The remaining 227 aa of the skate protein were 48% identical and 67% similar to human *KCNMB2*. Like the skate, alignment of the whale shark (*R. typus*) KCNMB2 showed 40 additional amino acids at the N terminus. Local alignment of the skate and whale shark proteins showed 56% identity and 72% similarity over 270 aa. *KCNMB2* from a chimera, the elephant shark (*C. milii*), was 54% identical and 69% similar over 227 aa, an alignment length similar to human. As the N terminus ball-and-chain domain of human *KCNMB2* is responsible for fast inactivation of B_K channels, the longer skate and whale shark N terminus may be functional. Further research should include confirmation by cloning and studies of how the gating behavior of skate B_K channels is modified by β-subunits.

16. Optogenetic Interrogation of Neural Inhibition with Light-Regulated $GABA_A$ Receptors.

WAN-CHEN LIN, MING-CHI TSAI, CHRISTOPHER M. DAVENPORT, and RICHARD H. KRAMER, *Department of Molecular and Cell Biology, University of California Berkeley, Berkeley, CA 94720*

Optogenetics is a hybrid approach that integrates optical and genetic manipulations of a biological target for precise and specific control in a complex system. Here we present the development and applications of optogenetic tools for unlocking the functional diversity of inhibitory neurotransmission in the brain. $GABA_A$ receptors ($GABA_A$Rs), a family of ligand-gated chloride channels, are major mediators of neural inhibition. A $GABA_A$R typically comprises two α, two β, and one tertiary subunit (γ or δ), with the α-subunit primarily determining its function and distribution. The expression profile of $GABA_A$Rs is highly diverse across different cell types, brain regions, and developmental stages. The functional rationales underlying this tremendous diversity remain elusive. To unravel the roles of individual subtypes in various brain functions, we have employed a chemical-genetic approach to engineer light-regulated $GABA_A$ receptors (LiGABARs) for all six α-isoforms, providing a comprehensive toolkit for optogenetic interrogation of neural inhibition. These receptors are bi-stable devices: they can be rapidly and reversibly switched between normal and antagonized states with two different wavelengths of illumination, and their functional states are preserved in darkness following a brief flash of conditioning light. The engineered receptors can be introduced into neurons exogenously via viral transduction or endogenously in knock-in mice. LiGABARs have been successfully implemented in brain slices and in vivo, enabling optical manipulation of inhibitory synaptic responses, neuronal firing, and network

activities in the mouse brain. We have employed this toolkit to functionally probe the differential contribution of a1-$GABA_A$Rs to distinct classes of inhibitory synapses in the mouse visual cortex. Moreover, we have also revealed the unique actions of α5-$GABA_A$Rs in regulating hippocampal long-term plasticity. These results demonstrate the unprecedented potential of LiGABAR-based optogenetics in decoding inhibitory neurotransmission underlying brain physiology and disorders.

17. Decreased Ca^{2+} Signaling, Ca_V1 Channel Expression, and Activity in Pancreatic β Cells Expressing Truncated DISC1 (Disrupted in Schizophrenia 1).

PING LU,[1] RICKY DAVID JONES,[1] RONGHUA ZHUGE,[1] ANN R. RITTENHOUSE,[1]* and AGATA JURCZYK,[2]* *[1]Department of Microbiology and Physiological Systems and [2]Diabetes Center of Excellence, University of Massachusetts Medical School, Worcester, MA (*Senior Co-authors)*

Schizophrenics often suffer from non-obese type 2 diabetes (T2D) in addition to cognitive impairment. In this study, we interrogated whether similar cellular changes occur in pancreatic β cells as found in neurons from a mouse model of schizophrenia. Specifically, we tested whether β cells exhibit disrupted excitation–secretion coupling when a truncated human DISC1 (*th*DISC1) gene, originally discovered in a Scottish family having high penetrance for schizophrenia (St Clair et al. 1990. *Lancet.* 336:13–16), is expressed selectively in mouse β cells upon ingesting doxycycline (+DOX). Previously, we found that expression of *th*DISC1 significantly decreases blood insulin levels and glucose-stimulated insulin secretion (GSIS) from isolated islets, revealing an independent role for DISC1 in β cells (Jurczyk et al. 2015. *FASEB. J.* 30:983–993). In central neurons, DISC1 regulates Ca_V2 channel expression, Ca^{2+} influx, and transmitter release (Tang et al. 2016. *Front. Synap. Neurosci.* http://dx.doi.org/10.3389/fnsyn.2016.00015). To determine whether DISC1 regulates Ca^{2+} physiology in β cells, we tested *th*DISC1 DOX+ versus DOX- β cells for changes in (*i*) intracellular Ca^{2+}signaling using Fluo 3-AM; (*ii*) Ca_V1 channel expression, the dominant channel class controlling GSIS, using immunohistochemistry; (*iii*) and Ca_V1 channel activity using whole-cell recording methods. We found increases in $[Ca^{2+}]_i$ following 20 mM glucose were delayed >2 min and diminished ~50% in dissociated β cells expressing *th*DISC1. When sections of pancreas were stained with an anti-$Ca_V1.2/1.3$ antibody (NeuroMab), *th*DISC1 DOX+ versus DOX- insulin-positive cells exhibited significant loss of staining. Consistent with lower functional Ca_V1 expression, both peak inward Ba^{2+} current, and FPL 64176–induced long-lasting L-type tail current decreased significantly in *th*DISC1 DOX+ (56 and 67%, respectively) versus DOX- β cells. No obvious change in activation kinetics was observed. These changes in excitation-secretion coupling mirror those in neurons expressing truncated DISC1. Thus, at the cellular level, truncated DISC1 precipitates disorders of secretion.

18. Visualizing the Arterial Wall Water Permeability Barrier with Femtosecond CARS Microscopy.

BERTRAND M. LUCOTTE,[1] CHLOE POWELL,[2] JAY R. KNUTSON,[3] KIM A. DORA,[2] and ROBERT S. BALABAN,[1] *[1]Laboratory of Cardiac Energetics, National Heart Lung and Blood Institute, National Institutes of Health, Bethesda, MD; [2]Vascular Pharmacology Research Group, Department of Pharmacology, University of Oxford, Oxford, UK; [3]Laboratory of Optical Spectroscopy, National Heart Lung and Blood Institute, National Institutes of Health, Bethesda, MD*

A large arterial transmural pressure gradient is essential to the normal physiological function of the circulation and indicates the presence of a significant barrier to water permeation. The nature of this barrier has generated high research interests since increases in arterial wall water permeability are associated with many diseases, including diabetes, atherosclerosis, acute shock and inflammatory conditions, and the aging process. Despite the importance of this fundamental arterial wall property in vascular function and drug pharmacodynamics, the precise location and biochemical composition of the arterial wall water barrier is unknown. Many have suggested this barrier is formed by the endothelial cell (EC) layer based on the effect of its gross mechanical disruption.

Using coherent anti-Stokes Raman scattering (CARS) microscopy and isotopic perfusion, we directly map the water permeability, and the associated pressure profile, in in vitro intact and pressurized rat mesenteric arteries (Lucotte et al. 2017. *Proc. Natl. Acad. Sci. USA.* 114(18): 4805–4810). Unlike most metabolites found in biological tissues, water CARS imaging at the OH stretch resonance is best achieved with femtosecond rather than picosecond excitation pulses. The signal thus generated is increased by two orders of magnitude while retaining isotopic specificity.

We identify the endothelial basolateral membrane as the major arterial water barrier and show that the apical membrane is highly permeable. This is confirmed by the endothelial distribution of the AQP1 water channel, which is found exclusively at the apical membrane. The advantageously positioned water barrier permits (1) arterial pressure to equilibrate within the EC cytosol and (2) direct arterial pressure transfer to the supporting basement membrane and internal elastic lamina macromolecules with minimal EC deformation. This transfer of pressure thus provides the mechanically sensitive EC with a protective mechanism against the recurring arterial pressure waves. Its disruption could contribute to EC dysfunction in various pathologies.

19. Effects of Peptidoglycan on Localization and Function of TonB in *Escherichia coli*. ARITRI MAJUMDAR, SALETE M. NEWTON, and PHILLIP E. KLEBBA, *Department of Biochemistry and Molecular Biophysics, Kansas State University*

Iron uptake through Gram (-) bacterial outer membrane (OM) proteins occurs by active transport that requires energy-transducing activity of the inner membrane (IM) protein, TonB. OM transporters like FepA bind ferric complexes and import them as a result of protein–protein interactions with TonB, which undergoes energy-dependent motion in the IM. TonB also binds the peptidoglycan (PG) beneath the OM, which organizes biogenesis/physiology and defines cell shape. This fact may explain restricted distribution of TonB to the central region of Gram (-) cells. We microscopically observed fluorescently labeled FepA and TonB proteins in vivo and correlated these images with radioisotopic iron uptake experiments in normal *E. coli* and in cells with compromised, degraded PG. The results showed that as PG was enzymatically degraded from within, GFP-TonB lost its restricted, central localization, and uniformly distributed throughout the IM. Degradation of PG reduced or abrogated uptake of [^{59}Fe]enterobactin through the OM, without compromising the uptake of [^{14}C]lactose through the IM, despite the fact that both transport reactions depend on electrochemical protonmotive force (PMF). These data revealed that restricted distribution of TonB in the central part of the cell, and its exclusion from the poles of the cell, derives from its association with the PG polymer in the bacterial periplasm. Furthermore, degradation of PG undermines TonB-dependent transport of ^{59}Fe through the OM, suggesting that associations between TonB and PG are mechanistically crucial to the function of OM metal transporters like FepA. We interpret the results in the context of the ROSET model of TonB-dependent active iron OM transport, which postulates low-affinity binding of the TonB C terminus to both PG and OM proteins, which, in combination with PMF-driven rotational motion, facilitates mechanical energy transfer from IM to OM. These reactions allow active transport in a bilayer that cannot sustain an ion gradient.

20. The Thresholds of an Excitable Signal Transduction Network Determine Cell Migratory Modes. YUCHUAN MIAO,[1] SAYAK BHATTACHARYA,[2] MARC EDWARDS,[3] HUAQING CAI,[4] TAKANARI INOUE,[3] PABLO A. IGLESIAS,[2,3] and PETER N. DEVREOTES,[3] *[1]Department of Biological Chemistry, School of Medicine, Johns Hopkins University, Baltimore, MD 21205; [2]Department of Electrical and Computer Engineering, Whiting School of Engineering, Johns Hopkins University, Baltimore, MD 21205; [3]Department of Cell Biology and Center for Cell Dynamics, School of Medicine, Johns Hopkins University, Baltimore, MD 21205; [4]State Key Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China.*

The diverse migratory modes displayed by different cell types are generally believed to be idiosyncratic. Here, we show that the migratory behavior of *Dictyostelium* was switched from amoeboid to keratocyte-like and oscillatory modes by synthetically decreasing PIP2 levels or increasing Ras/Rap-related activities. The perturbations at these key nodes of an excitable signal transduction network initiated a causal chain of events: The threshold for network activation was lowered, the speed and range of propagating waves of signal transduction activity increased, actin-driven cellular protrusions expanded, and, consequently, the cell migratory mode transitions ensued. Conversely, innately keratocyte-like and oscillatory cells were promptly converted to amoeboid by inhibition of Ras effectors with restoration of directed migration. We propose a unifying theory of how and why cells move, which might explain migratory transitions found in development as well as in pathological conditions.

21. In Vivo AO-SLO/OCT Imaging of Microglia During the Initiation and Resolution of Retinal Degeneration. ERIC B. MILLER, PENGFEI ZHANG, EDWARD N. PUGH, JR. and MARIE E. BURNS

Microglia, the resident macrophages of the central nervous system, are thought to be highly dynamic ramified cells that constantly surveil their microenvironment to remove neuronal debris and prune synaptic contacts. Using custom, high-resolution dual scanning laser ophthalmoscopy (SLO) and optical coherence tomography (OCT) systems with and without adaptive optics (AO), we have developed a highly repeatable in vivo preparation that allows high-resolution visualization of microglial activation, migration, and de-activation in response to localized, transient neuronal damage. In healthy retina, AO-SLO time-lapse imaging of GFP-expressing microglia (*Cx3CR1$^{+/GFP}$* mice) revealed strikingly little movement of the primary microglial branches, even 1 hour after an infrared light exposure that produced a spot (~150 mm) of acute damage to rod photoreceptors, as determined from changes in OCT light-scattering in the outer nuclear layer. However, over the course of days following the damaging exposure, microglia lost their branched structure and migrated in 3D to the region of damage, densely populating the area while other microglia only a few hundred micrometers away remained highly ramified and stationary. Gradually, microglia at the damage locus returned to their normal ramified morphology and mosaic distribution after 2 weeks. The return of microglia to their normal state coincided with the disappearance of light scattering changes associated with damaged rods. These results demonstrate ramified microglia in both healthy and damaged retina are relatively stationary rather than highly dynamic and that dramatic functional variation in subsets of microglial cells depend on their specific

location within the CNS. Furthermore, the ability to image both microglial activation, migration and resolution in vivo opens the door to visualizing the signaling mechanisms underlying these dramatic cellular transformations that are thought to be central for synaptic plasticity and maintenance, as well as mitigation of neurodegenerative diseases.

22. Stoichiometry of Kv2.1/Kv6.4 Heterotetramers Revealed by Fluorescent Single Channel Subunit Counting. LENA MOELLER,[1] GLENN REGNIER,[2] ALAIN J. LABRO,[2] DIRK J. SNYDERS,[2] and RIKARD BLUNCK,[3] *[1]Department of Biochemistry, Université de Montréal, Montreal, Canada; [2]Biomedical Sciences, University of Antwerp, Antwerpen, Belgium; [3]Department of Physics, Université de Montréal, Montreal, Canada*

It was previously shown that electrically silent voltage-gated channel subunits (KvS) do not form functional homotetramers but can assemble as heteromeric Kv2/KvS channels with rendered biophysical properties compared with Kv2 homotetramers. So far, only few of these heteromeric channels have been studied regarding their stoichiometry. FRET measurements have shown that for example Kv2.1/Kv9.3 channels have a fixed stoichiometry of 3:1 (Kerschensteiner et al. 2005. *Proc. Natl. Acad. Sci.* 102(17):6160–6165). In order to assess whether other heteromeric Kv2.1/KvS channels also adopt this arrangement of subunits, we previously investigated the Kv2.1/Kv6.4 heteromer by examining the functional channel properties of different concatemers. We found that the stoichiometry in these heterotetramers can be either 3:1 or 2:2. Here, we co-express Kv2.1 and a fluorescently tagged Kv6.4-GFP in *Xenopus* oocytes and verify the stoichiometry by single-molecule fluorescence imaging. Co-expressed Kv6.4-GFP is subjected to a fluorescence imaging. The number of fluorescently labeled subunits within a single heteromer can then be determined by counting the photobleaching steps observed as progressively each fluorescent label loses its fluorescence in a stepwise distinct event by photochemical destruction (Ulbrich and Isacoff. 2007. *Nature Methods.* 4(4):319–321). We confirm that there are maximally two Kv6.4 subunits per heteromeric complex. Furthermore, our results suggest that this arrangement of subunits is independent of the expression level of Kv6.4 subunits in the cell.

23. Finding Two-Photon Brighter Green Fluorescent Proteins in Obscure, Blue-Shifted Variants. ROSANA S. MOLINA,[1] MERRILEE THOMAS,[1] TAM M. TRAN,[2] GERARD G. LAMBERT,[3] ANYA SALIH,[4] ROBERT E. CAMPBELL,[2] NATHAN C. SHANER,[3] THOMAS HUGHES,[1] and MIKHAIL DROBIZHEV,[1] *[1]Department of Cell Biology and Neuroscience, Montana State University, Bozeman, Montana 59717; [2]Department of Chemistry, University of Alberta, Edmonton, Alberta T6G 2R3, Canada; [3]Scintillon Institute, San Diego, California 92121; [4]Western Sydney University, Penrith South DC, New South Wales 1797, Australia*

Two-photon microscopy together with genetically encoded fluorescent biosensors has facilitated deeper imaging of living tissue, especially the brain, leading to insights into physiological processes based on markers such as calcium activity and voltage changes across the membrane. Fluorescent proteins (FPs) and their physical properties are a critical consideration when designing new biosensors. Enhanced two-photon brightness (defined as the two-photon cross section multiplied by the fluorescence quantum yield) is particularly desirable because the high intensities of the two-photon excitation laser can cause tissue damage: with a brighter sensor, a lower laser power can be used for an equivalent signal. Genetic mutations within the beta barrel of an FP can cause the electrostatics around the chromophore to change, impacting both the one-photon and two-photon properties. Expanding on previous physical modeling of the anionic green FP (GFP) chromophore (Drobizhev et al. 2012. *J. Phys. Chem. B.* 116:1736–1744; Drobizhev et al. 2015. *Sci. Rep.* 5:13223), we derived a relationship that predicts a stronger two-photon maximum cross section with a blue-shifted one-photon absorption. In accordance with this prediction, we measured 12 blue-shifted FPs with this chromophore and found that they are up to two and half times brighter than the commonly used enhanced GFP, whereas the red-shifted but one-photon bright mNeonGreen is two times dimmer (Molina et al. 2017. *J. Phys. Chem. Lett.* 8:2548–2554). Our model and experimental data suggest that FPs should be optimized independently for the two-photon regime. Progress is underway to screen for even brighter blue-shifted mutants.

24. Opsin 3–Mediated Regulation of Skin Pigmentation. LAUREN E. OLINSKI,[1] RANA N. ÖZDEŞLIK,[2] and ELENA OANCEA,[2] *[1]Department of Molecular Biology, Cell Biology and Biochemistry, Brown University, Providence, RI; [2]Department of Molecular Pharmacology, Physiology and Biotechnology, Brown University, Providence, RI*

Opsins—light-activated G protein–coupled receptors (GPCRs)—are crucial junctions bridging photostimulation with internal signaling events. As photoreceptors, the majority of vertebrate opsins have been extensively characterized (Terakita. 2005. *Genome Biol.* 6:213), yet the functionality of extraocular opsin 3 (OPN3) remains to be elucidated. OPN3, first discovered in the mammalian brain (Blackshaw and Snyder. 1999. *J. Neurosci.* 19:3681–3690), is different from other vertebrate opsins in that it is expressed in several peripheral tissues, many of which are not readily exposed to light (Halford et al. 2001. *Genomics.* 72:203–208), and possesses a long carboxy terminus with no homology to other GPCRs. We recently discovered that OPN3 is highly expressed in

human epidermal melanocytes (HEMs; Haltaufderhyde et al. 2015. *Photochem. Photobiol.* 91:117–123), the melanin-producing cells of the skin. We found that reducing OPN3 expression levels in HEMs leads to an increase in melanin production, linking OPN3 to melanogenesis. Intriguingly, in HEMs, OPN3 interacts with melanocortin-1 receptor (MC1R), a key regulator of the melanogenic pathway. Activation of MC1R, a G_as-coupled GPCR (Yamaguchi et al. 2007. *J. Biol. Chem.* 282:27557–27561), leads to increased cAMP levels, while OPN3, a predicted G_ai-coupled GPCR (Koyanagi et al. 2013. *Proc. Natl. Acad. Sci. USA.* 110:4998–5003), has the opposite effect on cAMP levels, suggesting that the mechanistic purpose underlying the interaction of OPN3 and MC1R may hinge on regulation of cAMP levels upstream of melanin production. While invertebrate and non-mammalian homologues of OPN3 have been shown to be activated by blue light (Koyanagi et al. 2013. *Proc. Natl. Acad. Sci. USA.* 110:4998–5003), we did not detect light-induced OPN3 signaling in HEMs, suggesting that in these cells OPN3 may function as a constitutively active receptor or is activated by very low levels of ambient light. Our data reveal a novel function for the poorly characterized human OPN3 in skin pigmentation.

25. Two-Photon Imaging of Neuronal Activity in Freely Behaving Mice Using a Miniature Fiber-Coupled Microscope with Active Axial Focusing. BARIS N. OZBAY, GREGORY L. FUTIA, MING MA, ETHAN G. HUGHES, DIEGO RESTREPO,* and EMILY A. GIBSON,* *University Of Colorado Denver, Aurora, CO; *Senior Co-authors*

We have designed and built a miniature two-photon fiber-coupled microscope (TP-FCM) with active axial focusing for imaging neuronal activity in behaving and freely-moving mice. The TP-FCM has a weight of ~4 g. A coherent fiber-bundle with a diameter of 0.5 mm is used as a lateral scanning relay for excitation and emission collection, allowing the use of bench-top scanning and detection optics with multiple fluorescent indicators. A miniature electrowetting tunable lens enables active axial focusing. Two versions of the TP-FCM are demonstrated. (1) A cortical imaging TP-FCM with a cylindrical imaging volume of 200-μm diameter × 180-μm depth, lateral resolution of ~1.8 μm, and axial resolution of ~10 μm, designed for imaging through a glass cranial window. (2) A deep-brain TP-FCM designed, with an imaging volume of ~150-μm diameter × 100-μm depth, lateral resolution of ~1.4 μm, and axial resolution of ~8 μm, designed for imaging through a 0.5-mm diameter GRIN lens. We show neuronal activity recorded from the motor cortex neurons expressing GCaMP6s of freely behaving mice. The TP-FCM design allows for the recording of neuronal activity from a large three-dimensional volume with the ability to rapidly change axial focus and execute more arbitrary volumetric scan trajectories.

26. Distance-Resolving Voltage Clamp Fluorometry: Ångström-Precision Measurements of Membrane Protein Structural Dynamics Under Physiological Conditions. ANTONIOS PANTAZIS and RICCARDO OLCESE, *Division of Molecular Medicine, Department of Anesthesiology and Perioperative Medicine, David Geffen School of Medicine, University of California, Los Angeles*

The holy grail of structural biology, i.e., the simultaneous quantitative determination of a protein's structure and function, remains very difficult to attain. Voltage clamp fluorometry (VCF) is an optical approach for characterizing the functional properties of fluorescently labeled domains of ion channels and related proteins. We adapted both experimental and theoretical aspects of VCF, endowing it with the ability to accurately measure intramolecular distances up to 2.5 nm with a very fine grain. We call the new approach distance-resolving VCF (drVCF).

Using drVCF on conducting human BK channels expressed in oocytes under voltage clamp, we found that the transmembrane helix S4 of the voltage-sensing domain (VSD) is virtually equidistant from juxtaposed helices S1 and S2 in the Active state (mean, [95% confidence intervals]; S1–S4: 16.5 [15.5,17.5] Å; S2–S4: 15.9 [15.1,17.5] Å). These measurements are in excellent agreement with the open BK channel structure recently resolved by cryo-EM (Tao et al. 2017. *Nature.* 541:46–51; S1–S4 and S2–S4 distance ~16 Å). We also found that, in the resting state, S4 is nearer to S1 and S2 (7.5 [5.7,9.3] and 7.1 [6.0,8.6] Å, respectively). This information on the rearrangement of S4 with respect to S1 and S2 provides the structural correlate for depolarization-evoked BK channel activation and, as it was acquired from functional, conducting channels, an ideal complement to BK functional studies.

To validate drVCF, we used synthetic polyproline-based peptides, initially employed by Stryer and Haugland to validate FRET. In peptides with 2, 4, or 7 prolines, drVCF length estimates were off, on average, by 0.99 ± 0.24 Å, also providing a good estimate for the per-residue rise of a polyproline type II helix (drVCF: 3.4 [3.0,3.7] Å; actual: 3.1 Å). Thus, drVCF is a remarkably accurate optical approach for the real-time measurement of intramolecular distances and distance changes under physiologically relevant conditions, simultaneously with protein function.

27. A Photoconvertible Genetically Encoded Glutamate Highlighter for Astrocyte Imaging. STELIOS PAPADOPOULOS,[1] GRACE OR,[1] JENNIFER JAHNCKE,[2] CHUNYANG DONG,[1] JASON LAMBERT,[2] KAREN ZITO,[2] and LIN TIAN,[1] *[1]Biochemistry and Molecular Medicine, School of Medicine, University of California, Davis, Davis, CA; [2]Center for Neuroscience, University of California, Davis, Davis, CA*

In the last decade, astrocytes have emerged as key participants in brain development and function in health and disease (Volterra and Meldolesi. 2005. *Nat. Rev. Neursci.* 6(8):626–640). Astrocytes have shown to play a crucial role in synaptogenesis and synaptic plasticity (Perea et al. 2009. Cell. 32(8):421–431) by responding to neurotransmitters (Porter and McCarthy. 1996. *J. Neurosci.* 16(16):5073–5081) and by releasing gliotransmitters (Araque et al. 2014. *Cell.* 81(4):728–739). However, the mechanisms through which astrocytes interact with neurons and contribute to the structural and functional plasticity of the brain remain poorly understood (Haber et al. 2006. *J. Neurosci.* 26(35):8881–8891; Clarke and Barres. 2013. *Nat. Rev. Neurosci.* 14(5):311–321). Recently, improved genetically encoded indicators of neuronal activity have allowed for functional measurements through optical recordings of calcium (Tian et al. 2009. *Nat. Methods.* 6(12):875–881) and glutamate (Marvin et al. 2013. *Nat. Methods.* 10(2):162–170). These applications have significantly advanced the field of systems neuroscience by permitting optical recordings in neurons, but have been inadequate in the context of astrocytes. The reason for this is that until recently we lacked an optical approach that can provide both a super-resolution image of the subcellular distribution of astrocytic compartments and a functional readout. Therefore, we have developed a new class of genetically encoded photoconvertible glutamate highlighters that permit functional recordings of glutamate transients and post-hoc analysis of the distribution of glutamate transporters, correlating functional plasticity with structural plasticity. We demonstrate the utility of this new glutamate highlighter in primary astrocyte neuron co-cultures. Ultimately, we expect this tool to bridge the knowledge gap of the constant interplay between neurons and astrocytes contributing to the plasticity of the brain.

28. Imaging Neuromodulatory Signaling With Genetically Encoded Indicators. TOMMASO PATRIARCHI,[1] KOHEI J. SEKIGUCHI,[2] AARON MARLEY,[3] BRIAN P. MCGREW,[1] JOHN T. WILLIAMS,[4] MARK VON ZASTROW,[3] AXEL NIMMERJAHN,[2] and LIN TIAN,[1] *[1]Biochemistry and Molecular Medicine, University of California, Davis, Davis, CA; [2]Waitt Advanced Biophotonics Center, Salk Institute for Biological Studies, La Jolla, CA; [3]Department of Psychiatry, Department of Cellular and Molecular Pharmacology, University of California, San Francisco, San Francisco, CA; [4]Vollum Institute, Oregon Health and Sciences University, Portland, OR*

Neuromodulation is a key feature of all nervous systems (Marder. 2012. *Neuron.* 76:1–11). Neuromodulators such as dopamine, norepinephrine, or serotonin exert powerful control over neural circuit dynamics linked to, for example, arousal, attention, emotion, or cognitive perception. Altered neuromodulator signaling is a key feature of virtually all human neurological and psychiatric disorders, including Parkinson's disease, schizophrenia, depression, and addiction (Tye and Deisseroth. 2012. *Nat. Rev. Neurosci.* 13:251–266). We address the need for large-scale in vivo optical measurement of neuromodulator signaling by developing a toolbox of genetically encoded indicators based on circularly permuted GFP for a variety of neuromodulators including dopamine, norepinephrine, and serotonin. We have focused on characterizing the sensitivity, affinity, and specificity of these sensors in detecting dopamine and norepinephrine in mammalian cells and in dissociated neurons. In addition, we further tested the performance of these sensors in acute brain slices and in behaving mice with regard to their ability to detect stimulated or behaviorally evoked monoamine release. We expect these new tools to be broadly applicable, permitting sensitive measurement of neuromodulator signaling at synaptic, cellular, and system levels, furthering our understanding of fundamental neural circuit function.

29. Source-Localized Multifocal Two-Photon Microscopy for High-Speed Functional Imaging. PETER QUICKE,[1,4] MARK NEIL,[2,4] THOMAS KNÖPFEL,[3,4] SIMON R. SCHULTZ,[1,4] and AMANDA J. FOUST,[1,4] *[1]Department of Bioengineering, [2]Department of Physics, [3]Department of Medicine, [4]Centre for Neurotechnology, Imperial College London*

We have optimized a multifocal two-photon microscope (MTPM) for high-speed functional imaging of neuronal activity. Accurately reconstructing underlying cellular dynamics from recorded fluorescence indicator traces requires high temporal and spatial resolution imaging. Two-photon point scanning (TPPS) approaches allow deep imaging in scattering tissue like the brain but are limited in temporal resolution by the need to serially acquire each image pixel. Parallelizing this acquisition by using multiple foci allows a large increase in speed whilst fully sampling the field of view. The need to image the resulting fluorescence, however, means that this technique is vulnerable to image blur by emission photon scattering. We have implemented a spatially sparse MTPM that can resolve cellular-resolution calcium transients in adult mouse brain slices bulk loaded with Cal-520. Imaging at 200 frames per second with a 110 × 200–μm field of view, the system resolves calcium transients with a signal to noise ratio of 10 ± 2 dB. We have also implemented image reconstruction algorithms that take advantage of the structured illumination in our sample to reduce the effect of scattering and we quantify the performance improvement in terms of signal localization, depth penetration, and contrast enhancement. We assess our system performance compared with TPPS and wide field single photon imaging. We also discuss the limiting factors of the system, in particular imaging depth and available laser power,

which will determine the broader applicability of this technique.

30. Relationship Between Different Short-Term Plasticity Mechanisms at an Identified Synapse.
MICHELLE A. SÁNCHEZ-RIVERA, MONTSERRAT G. CERCÓS, and CITLALI TRUETA, *Departamento de Neurofisiología, Instituto Nacional de Psiquiatría Ramón de la Fuente Muñiz, Mexico City, Mexico*

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.
MASOUD SEPEHRI RAD,[1] LAWRENCE B. COHEN,[1,2] O. BRAUBACH,[1] and BRADLEY J. BAKER,[1,3] *[1]Center for Functional Connectomics, Brain Science Institute, Korea Institute of Science and Technology (KIST), Seoul, 02792, Korea; [2]Department of Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, CT 06520; [3]Department of Neuroscience, Korea University of Science and Technology, Daejeon, South Korea*

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.
AMOL V. SHIVANGE,[1,2] AARON L. NICHOLS,[2] PHILIP M. BORDEN,[1] ARON KAMJAYA,[2] ANAND K. MUTHUSAMY,[2] JANICE H. JEON,[2] ELIZABETH K. UNGER,[3] LIN TIAN,[3] JONATHAN S. MARVIN,[1] LOREN L. LOOGER,[1] and HENRY A. LESTER,[1,2] *[1]Janelia Research Campus, Howard Hughes Medical Institute, Ashburn, VA; [2]Division of Biology and Biological Engineering, Caltech, Pasadena, CA; [3]Dept. of Biochemistry and Molecular Medicine, University of California at Davis*

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.

Support: NIH, NARSAD, California TRDRP, and HHMI.

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.

Supported by: US NIH Grants DC005259, DC016133, NS099691 and a Ruth L. Kirschstein National Research Service Award DC012981, Grant WCI 2009-003 from the National Research Foundation of Korea, and a James Hudson Brown–Alexander Brown Coxe Fellowship from Yale University.

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

capable of acquiring large fields of view with high spatiotemporal resolution. While conventional light sheet microscopes utilize two or more orthogonal objectives for their excitation and detection paths, SCAPE microscopy accommodates both light paths within a single high numerical aperture objective. This geometry permits SCAPE to circumvent optomechanical limitations that constrain both sample selection and 3D imaging speed in conventional light sheet systems, while delivering similar advantages, including low phototoxicity and relatively simple instrumentation. As a result, SCAPE can deliver these benefits, and high 3D imaging speeds to application spaces more usually met by laser-scanning techniques such as confocal and two-photon microscopy.

Thus far, we have evaluated SCAPE microscopy's ability to image a range of intact, behaving organisms including freely crawling *Drosophila* larvae, the whole brain of adult, behaving *Drosophila*, the whole larval zebrafish brain, *C. Elegans* worms and the awake, behaving mouse brain. With each application we have developed optimized experimental methods and system configurations to meet requirements, including adaptable fields of view, volume rates, depths of field, spatial resolution, and spectral multiplexing.

Here, we will describe the development of these second generation SCAPE systems, including a simplified design that offers much more versatility and better performance than our first-generation system. In addition to a larger, more uniform field of view with better resolution, these improvements have yielded volumetric imaging speeds up to and exceeding 100 Hz.

We will showcase the system's ability to adapt to the unique requirements of different model organisms and present our latest neuroimaging results.

36. Voltage Imaging from Dendritic Spines Using Patterned Illumination Based on Computer-Generated Holography (CGH). JU-YUN WENG,[1] DIMITRII TANESE[2], VALERIA ZAMPINI,[2] VINCENT DE SARS[2], MARCO CANEPARI,[3,4,5] BALAZS ROZSA,[6] VALENTINA EMILIANI,[2] and DEJAN ZECEVIC,[1] *[1]Department of Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, CT 06520; [2]Neurophotonics Laboratory, CNRS UMR8250, Paris Descartes University, 75270 Paris, France; [3]Laboratory for Interdisciplinary Physics, UMR 5588, Université Grenoble Alpes and CNRS, 38402 Saint Martin d'Hères, France; [4]Laboratories of Excellence, Ion Channel Science and Therapeutics, France; [5]Institut National de la Santé et Recherche Médicale (INSERM), France; [6]Institute of Experimental Medicine of the Hungarian Academy of Sciences, Budapest H-1083, Hungary*

Additional research is necessary to clarify electrical role of dendritic spines. Notwithstanding decades of theoretical and modeling studies, as well as calcium, sodium, and voltage imaging experiments and nanoelectrode recordings, the elementary question of the value of spine neck resistance relative to the input impedance of thin spiny dendrites is controversial. This controversy allows for different hypotheses about electrical role played by dendritic spines. To obtained direct evidence, we previously used an electrochromic voltage-sensitive dye (JPW3028) as a transmembrane optical voltmeter with a linear scale capable of recording simultaneously electrical signals from individual spines and parent dendrites. The results showed that the spine neck does not electrically isolate synapses on spines. Thus, synapses on spine heads behave as if they are located directly on dendrites. However, scattered light and photodynamic damage currently limit the investigation of more complex electrical phenomena that require many repeated measurements from dendritic spines. Here, we demonstrate a dual-color computer generated holography (CGH) illumination system, which was used for multisite optical recording and glutamate uncaging. The specific intensity-graded illumination patterns were designed to eliminate contamination of spine signals by the scattered light from parent dendrites thereby increasing the effective spatial resolution of voltage imaging. Additionally, the restriction of the high-intensity illumination exclusively to regions of interest (spine heads and small sections on parent dendrites) can significantly reduce photodynamic damage. Furthermore, in parallel with voltage imaging, we successfully used a second CGH system for multisite glutamate uncaging. Overall, a dual-color CGH illumination system drastically improved the spatial resolution of voltage imaging, reduced photodynamic damage, and provided true simultaneous multiple site uncaging of glutamate.

37. Developing an Optogenetic Tool for Tethering Mitochondria and Endoplasmic Reticulum in Neurons. MASAYUKI YAZAWA and FAN SHI, *Department of Rehabilitation and Regenerative Medicine, Columbia Stem Cell Initiative, Columbia University, New York*

Interaction between mitochondria and endoplasmic reticulum (ER) is essential for calcium handling, metabolism, and autophagy. Here, we present novel light-induced systems to control the interaction between mitochondria and ER in mammalian cell lines and primary neurons. To develop this system, first we have tested and optimized multiple light-induced heterodimer domains in mammalian cell lines, HEK 293T and NIH 3T3 cells. Using electron and confocal microscopes with the optimized systems, next we confirmed that blue light (~470 nm, shot illumination ~1 sec) could induce tethering of ER with mitochondria immediately. In addition, we developed reversible and non-reversible types of light-induced systems for tethering mitochondria and ER for further applications in primary neurons. This new optogenetic tool will help us study the

molecular mechanisms underlying neuronal calcium signaling, metabolism, and autophagy.

38. Synaptic Targeting Genetically Encoded Voltage Indicators. SHENG ZHONG, DOUGLAS STORACE, and LAWRENCE B.COHEN, *Dept. of Cellular and Molecular Physiology, Yale University School of Medicine*

Genetically encoded voltage indicators (GEVIs) offer direct measurement of neuron membrane potential changes with fluorescence changes. While the molecular mechanism of GEVIs were explored (Storace et al. 2016. *Trends Neurosci.* 39(5):277–289), further improvements on spatial and temporal resolution are still highly desired. GEVIs can express well on neuronal cell membrane, but the fluorescence in unwanted region would raise up background noise. For more accurate measurement, we generate synaptic targeting constructs of Arclight for mammalian neuron and zebrafish neurons, respectively, with postsynaptic-interacting protein. The synaptic targeting GEVI can give quantal resolution imaging of synaptic transmission in cultured cells and transgenic animals that can discover short-term synaptic plasticity and locomotor behavior.

Additional Abstracts

39. Co-translational regulation of potassium and sodium channel expression mediated bymRNA interaction. CATHERINE A. EICHEL, ERICK RÍOS-PÉREZ, FANG LIU, DAVID K. JONES and GAIL A. ROBERTSON, *Department of Neuroscience, University of Wisconsin School of Medicine and Public Health, Madison, WI.*

The balance of the ion currents that generate the cardiac electrical impulse is crucial for proper heart function. Perturbation in the expression of either the rapid delayed rectifying potassium channel gene (KCNH2) or the sodium channel gene (SCN5A) can result in dramatic cardiac arrhythmias and sudden death, but how cardiac cells coordinate expression of these channels is unknown. We recently demonstrated that alternate transcripts from the KCNH2 gene encoding hERG1a and 1b subunits are physically associated. This allows a proximity that promotes co-translational assembly and ensures proper subunit constituency. Here, we report that hERG transcripts are also associated with SCN5A mRNAs using single-molecule fluorescence in-situ hybridization (smFISH) in iPSC-CMs. RT-PCR after immunoprecipitation of either hERG or NaV1.5 channels has been used to determine the extent of this macromolecular association. We found association of hERG1a, hERG1b, and SCN5A mRNAs and their corresponding proteins hERG1a, hERG1b and NaV1.5, indicative of a co-translational complex. smFISH in combination with immunofluorescence revealed that 45% of the actively translated hERG1a transcripts are associated with SCN5A mRNAs. Of those complexes, 70% were located within 10 ?m from the nucleus, consistent with a distribution in active protein synthesis domains of the endoplasmic reticulum. Furthermore, the transcripts can be co-regulated in iPSC-CMs as indicated by a coordinate decrease of SCN5A, hERG1a and hERG1b transcript levels upon hERG1b-specific silencing. Whole-cell patch clamp revealed a corresponding reduction in the magnitude of the potassium IKr and sodium INa currents. The co-translational association and co-regulation of transcripts may represent a general mechanism by which cardiac cells coordinate expression and thus activity of different ion channel types. This mechanism may shed new light on cardiac rhythm maintenance and arrhythmogenic disorders, as well as other ion channel-related diseases.

40. Suppressive effects of amyloid peptide fragments 1-42 and the toxic “core” 25-35 on Kv1.1 channel activity. JOSEPH FARLEY, KRISTI DEBOEUF, MOHAMMAD ISLAM, and NICK THELEN, *Neuroscience, Indiana University, Bloomington, IN 47405*

Past studies have shown that Aβ’s role in the pathogenesis of Alzheimer’s disease (AD) involves disruption of Ca2+ homeostasis and synaptic communication, impairment of long-term potentiation (LTP), and eventual neurotoxicity. But the mechanisms underlying these effects remain unclear. Work in our lab suggests that Aβ-inhibition of voltage-dependent K+ channels (e.g., Kv1.1) activity is among the earliest steps. Using murine Kv1.1 channels expressed in Xenopus oocytes, we previously elucidated a pathway in which Ca2+-dependent activation of PP2B, PKC, PTKs, and RhoA all participated to produce rapid strong suppression of Kv1.1 activity. Because Kv1.1 and related channels are activated during an action potential, regulate Ca2+ influx, and inhibition of Kv1-family channels can be neurotoxic, we have hypothesized that Aβ-suppression of Kv1 channels could contribute to AD pathology. We assessed the effects of the Aβ(1-42) peptide (monomers and low n-oligomers) and the core fragment [Aβ(25-35)] peptide on murine Kv1.1 channels in oocytes. Bath application of Aβ(1-42) produced a concentration-dependent suppression of macroscopic Kv1.1 current: ~50% suppression within 30 m for 1 μM.. Aβ suppression of Kv1.1 was partially Ca2+- and PP2B-dependent, being reduced by ~50% when cells were loaded with BAPTA-AM, or exposed to the PP2B-inhibitor cyclosporine A (CsA). Patch-clamp results suggest that Aβ-suppression of Kv1.1 involves both PP2B-dephosphorylation and direct protein-protein interaction of Aβ with Kv1.1 channel

subunits. Exposure of inside-out single Kv1.1 channels in ripped-off oocyte patches to application of purified, catalytically-active PP2B produced gradual reductions in p(open), followed by abrupt disappearance of Kv1.1 activity. Application of Aβ to the intracellular face of Kv1.1 channels under conditions where little enzymatic activity could occur also produced dramatic reductions in p(open). Additional results indicate that 2 μM of Aβ(25-35) suppressed Kv1.1 currents by ~40%. Using "tip-dip" artificial membrane methods, 1 μM Aβ(25-35) exposure eliminated Kv1.1 channel activity when applied to the intracellular side.

41. Intrinsic Optical Signal (IOS) imaging is widely used non-invasive approach for monitoring brain activity. MAKI KOIKE-TANI, *Marine Biological Laboratory, Woods Hole, M 02543*.

Conventional IOS is monitored by changes of transmittance or scattering of light through the tissue without regard to the polarization of light. We included polarization signal in IOS by building a new instantaneous polarized light microscope. This microscope can detect sub-cellular birefringence signal, which reports structural changes of molecular orders such as cytoskeletons and membrane architectures in cytoplasm. Using mice hippocampal slice we observed static birefringence signal from stratum radiatum of area CA1, and analyzed that its optical slow axis orientation is perpendicular to the length of Schaffer collateral axons. We detected reproducible special and temporal patterns of birefringence change in response to nerve stimulation of Schaffer collateral (40 Hz, 50 pulses). This birefringence signal at stratum radiatum transiently increased and then recovered to the original level. The time constant of this signal recovery was 30 ± 2.3 sec (n=29), which was faster than that of transmittance light intensity change simultaneously monitored through conventional IOS imaging. These optical signals are inhibited by the application of ionotropic glutamate receptor blockers (CNQX and D-APV), and were completely abolished by the sodium channel blocker (TTX). These signals are greatly enhanced by application of the glutamate transporter inhibitor TBOA, but not by the inhibitor of glial glutamate transporter DHK. Those signals were not affected by the blocker of group I metabotropic glutamate receptor CPCCOEt. These results indicate that glutamatergic synaptic transmission is involved in the generation of birefringent signals. This activity dependent birefringent signal change can be induced by activity of postsynaptic glutamate receptors followed by transient structural changes in neurons, such as synaptic morphology, rearrangement of cytoskeletons, and remodeling of membranous structures.

42. Better (Neural Circuit) Labelling Through Chemistry. JULIAN NG[1,4,] IVAN CORREA[2], KATHRIN LANG[3], NACHIKET D. KASHIKAR[4], FRANCISCO PESTANA[4], SEBASTIAN CACHERO[5], BEN SUTCLIFFE[5], GREGORY JEFFERIS[1,5], AND MATTHIAS LANDGRAF,[1] *[1]Dept of Zoology, University of Cambridge; [2]New England Biolabs, Ipswich (USA); [3]Technische Universität München (Germany); [4]Department of Neuroscience, Janssen Research and Development, A Division of Janssen Pharmaceutica NV, Beerse, Belgium; [5]MRC Laboratory of Molecular Biology, Cambridge Biomedical Campus.*

Functional neural networks require diverse neuronal types to act together by forming synaptic connections and gating the neural information flow. One approach to its better understanding is to determine how synaptic connections arise from individual neurons connected to circuits. Such connectivity information can help to decipher the neurophysiological and behavioural properties governed by a given neural network. Over the past few years, we have developed genetically-encoded protein tags that incorporate small, sol-

uble chemical ligands and that can be synthetically coupled to bright, organic fluorophores. Through fluorescence microscopic studies, we have shown this ultrafast and even labelling method works when applied to Drosophila and mouse circuits in the brain, when investigating individual neuronal types and synaptic protein clusters (references below). Here we will present the latest iterations of this method and how it can be applied to determine neural circuit logic.

Sutcliffe B, Ng J, Auer TO, Pasche M, Benton R, Jefferis GSXE, and Cachero S. Second Generation Drosophila Chemical Tags: Sensitivity, Versatility and Speed. (2017) Genetics; DOI: https:doi.org/10/1534/genetics.116.199281

Kohl J, Ng J, Cachero S, Dolan MJ, Sutcliffe B, Tozer A, Ruehle S, Krueger D, Frechter S, Branco T, Tripodi M, Jefferis, GSXE. Ultra-fast tissue staining with chemical tags. (2014) Proc. Natl. Acad. Sci. USA; doi: 10.1073/pnas.141108711

43. Lattice light-sheet imaging of membrane nanotubes between human breast cancer cells in culture and in brain metastases. IAN PARKER[1,2], KATRINA T. EVANS[2], KYLE ELLEFSEN[1], DEVON A. LAWSON[2], AND IAN F. SMITH,[1] [1]*Department of Neurobiology and Behavior,* [2]*Department of Physiology and Biophysics.*

Cells have long been known to communicate using well-established mechanisms, such as through the secretion of soluble factors, membrane-associated proteins, extracellular vesicles, gap junction channels and synapses. A new form of cellular communication has been proposed based on the discovery of membrane nanotubes between mammalian cells (Rustom et al., 2004); long and thin extensions of the plasma membrane that relay specific and targeted messages between cells separated by tens of microns. Difficulties in visualizing and studying nanotubes within intact tissues have, however, prompted skepticism regarding their in vivo relevance, and most studies have been confined to cell culture systems. A number of recent studies have demonstrated the increasing role for membrane protrusions variously termed tumor microtubes (Jung et al., 2017; Osswald et al., 2015; Weil et al., 2017) and nanoscale bridges (Connor et al., 2015) in the pathogenesis and spread of cancerous tumors. However, the lack of a specific marker and imaging methodologies to visualize nanotubes has hindered their study within intact live tissues. Here, we introduce lattice light-sheet imaging of MDA-MB-231 human breast cancer cells genetically engineered to express membrane–targeted GFP as a promising approach to visualize membrane nanotubes in vitro and in situ. We demonstrate that cultured cells form multiple nanotubes that mediate intercellular communication of Ca2+ signals and actively traffic GFP-tagged membrane vesicles along their length. Furthermore, we directly visualize nanotubes in situ, interconnecting breast cancer cells in live acute brain slices from an experimental mouse model of breast cancer brain metastasis. This amenable experimental system should facilitate the transition of the study of intercellular communication by membrane nanotubes from cell culture to the whole animal.

Connor, Y., Tekleab, S., Nandakumar, S., Walls, C., Tekleab, Y., Husain, A., Gadish, O., Sabbisetti, V., Kaushik, S., Sehrawat, S., et al. (2015). Physical nanoscale conduit-mediated communication between tumour cells and the endothelium modulates endothelial phenotype. Nature communications 6, 8671.

Jung, E., Osswald, M., Blaes, J., Wiestler, B., Sahm, F., Schmenger, T., Solecki, G., Deumelandt, K., Kurz, F.T., Xie, R., et al. (2017). Tweety-Homolog 1 Drives Brain Colonization of Gliomas. J Neurosci 37, 6837-6850.

Osswald, M., Jung, E., Sahm, F., Solecki, G., Venkataramani, V., Blaes, J., Weil, S., Horstmann, H., Wiestler, B., Syed, M., et al. (2015).

Additional Abstracts

Brain tumour cells interconnect to a functional and resistant network. Nature 528, 93-98.

Rustom, A., Saffrich, R., Markovic, I., Walther, P., and Gerdes, H.H. (2004). Nanotubular highways for intercellular organelle transport. Science 303, 1007-1010.

Weil, S., Osswald, M., Solecki, G., Grosch, J., Jung, E., Lemke, D., Ratliff, M., Hanggi, D., Wick, W., and Winkler, F. (2017). Tumor microtubes convey resistance to surgical lesions and chemotherapy in gliomas. Neuro-oncology.

44. Wide-field optical mapping (WFOM) of large-scale neural activity and hemodynamics in the awake mouse cortex. MOHAMMED A. SHAIK, DAVID N. THIBODEAUX, SHARON H. KIM, ELIZABETH M.C. HILLMAN, *Columbia University in the City of New York*.

Wide-field mapping of cortical activity in the mammalian brain has historically been achieved using voltage sensitive dyes (VSDs) or so-called 'intrinsic signals' derived from changes in cortical hemodynamics. While effective for identifying functional regions, VSDs require cortical application, lack cellular specificity and suffer from low signal to noise, while intrinsic signals provide only a surrogate measure of neural activity, introducing spatiotemporal ambiguity.

The recent development of genetically encoded fluorescent calcium indicators provides a new way to approach large-scale recording of cortical neural activity in animal models. Cell type specific expression of GCaMP in transgenic mice, for example can enable longitudinal recording of cellular activity with high contrast and no need for the application endogenous dyes. While techniques such as two-photon microscopy are now commonly used to image intracellular calcium dynamics at the cellular level, wide-field optical mapping can instead measure neuronal activity over large regions of the cortex of the awake mouse brain.

We have developed a simple multispectral system for wide-field optical mapping(1) (WFOM) in awake, behaving mouse that enables real-time recording of neural activity over the entire dorsal surface of the cortex (through thinned skull) in the context of spontaneous behavior, task performance and perturbations such as delivery of drugs or the development of disease states. An important feature of this system is our use of multiple LED illumination wavelengths, interlaced to record both GCaMP fluorescence and multispectral diffuse reflectance to capture simultaneous cortical dynamics of oxy- and deoxy-hemoglobin. This parallel recording is necessary to correct fluorescence signals for the effects of hemoglobin on both fluorescence excitation and emission light. However, the system thus also permits large-scale dynamic analysis of neural activity and its pixelwise relationship to modulations in cortical blood flow. We are applying this multimodal WFOM system to study and model the contribution of different types of vascular endothelium-dependent vasoactivity to neurovascular coupling in the awake mouse brain. Our studies include pharmacological and disease-based perturbations to endothelial function and permit direct observation of the effects of these perturbations on neurovascular coupling, neuronal activity and behavior in real time. Additional studies include characterizing postnatal neurovascular development (2) and the role of brain-wide spontaneous neuronal activity in the 'resting-state' mouse (3). These studies are providing new insights for interpretation of hemodynamic signals detected in human functional magnetic resonance (fMRI) studies.

1. Y. Ma, M. A. Shaik, S. H. Kim, M. G. Kozberg, D. N. Thibodeaux, H. T. Zhao, H. Yu, E. M. Hillman, Wide-field optical mapping of neural activity and brain haemodynamics: considerations and novel approaches. Philosophical transactions of the Royal Society of London. Series B, Biological sciences

371, (2016); published online Epub-Oct 05 (10.1098/rstb.2015.0360).

2. M. G. Kozberg, Y. Ma, M. A. Shaik, S. H. Kim, E. M. Hillman, Rapid Postnatal Expansion of Neural Networks Occurs in an Environment of Altered Neurovascular and Neurometabolic Coupling. The Journal of neuroscience : the official journal of the Society for Neuroscience 36, 6704-6717 (2016); published online EpubJun (10.1523/JNEUROSCI.2363-15.2016).

3. Y. Ma, M. A. Shaik, M. G. Kozberg, S. H. Kim, J. P. Portes, D. Timerman, E. M. Hillman, Resting-state hemodynamics are spatiotemporally coupled to synchronized and symmetric neural activity in excitatory neurons. Proceedings of the National Academy of Sciences of the United States of America 113, E8463-E8471 (2016); published online EpubDec 27 (10.1073/pnas.1525369113).

45. Instantaneous imaging of biological strutures with weak birefringence using a new polychromatic polarized light microscope. MICHAEL SHRIBAK, *Marine Biological Laboratory, 7 MBL St., Woods Hole, MA, USA 02543*

A traditional polarized light microscope depends on the optical path difference between two polarization components of light to generate color. That is, as two beams of light pass through an object, they travel at different speeds, which throws them out of phase. If they recombine in a certain way, the waves generate color. But neurons and other cells are inherently weak optical structures, and that's always made them appear a monochromatic gray under the microscope's lens. New polychromatic polarized light microscope exploits a different aspect of polarized light called vector interference. Here's how it works: The microscope produces white light with orientation of the electric vector (or polarization angle) proportional to the wavelength and sends it to the sample. The light waves recombine, or interfere, as they pass through the sample. Because different colors interfere at different angles - such as those created by the shape of the neurons - we can generate a full spectrum of color from this otherwise monochromatic gray sample. We are already using the microscope, called a polychromatic polscope, in a range of studies (Shribak. 2015. Sci. Rep. 5:17340). It is used to investigate the pathology of cancer cells, suggesting it may hold potential as a high-resolution diagnostic tool. It can also be used to study disorders affecting blood cells, such as sickle cell disease and malaria. Also new microscope shows great promise as a mapping tool for neuroscientists. Not only does it image neurons at single-cell resolution, it shows how these cells interconnect. Such information is critical for learning more about how a normal brain works or about what might be going wrong with those interconnections during Alzheimer's disease and other neurological conditions.

46. Regulation of molecular alignment in protein assemblies in living cells. TOMOMI TANI, *Marine Biological Laboratory, 7 MBL St., Woods Hole, MA, USA 02543*

Regulation of molecular alignment drives the physiological functions of many protein assemblies in living cells yet remains difficult to explore accurately through space and time. We built an instantaneous fluorescence polarization microscope, which simultaneously images position and orientation of fluorophores in living cells with single-molecule sensitivity and a time resolution of 100ms (Mehta et al., 2016 Proc Natl Acad Sci USA 113 (42), E6352-E6361). We developed image acquisition and analysis methods to track single particles that interact with higher-order assemblies of molecules. We tracked the fluctuations in position and orientation of molecules

from the level of an ensemble of fluorophores down to single fluorophores. We tested our system in vitro using fluorescently labeled DNA and F-actin in which the ensemble orientation of polarized fluorescence is known. We then tracked the orientation of sparsely labeled F-actin networks at the leading edge of migrating human keratinocytes, revealing the anisotropic distribution of actin filaments relative to the local retrograde flow of the F-actin network. Additionally, we analyzed the position and orientation of septin-GFP molecules incorporated in septin bundles in growing hyphae of a filamentous fungus. Our data indicate that septin-GFP molecules undergo positional fluctuations within ~350 nm of the binding site and angular fluctuations within ~30° of the central orientation of the bundle. By reporting position and orientation of molecules while they form dynamic higher-order structures, our approach can provide new insights into how micron-scale ordered assemblies emerge from nano-scale molecules in living cells.

47. Impairment of Bergmann glia calcium activation by ethanol and consequences for local circuit activity. LIANG YE[1,3], MURAT ORYNBAYEV[1], Xiangyu Zhu[1,3] AND MARTIN PAUKERT,[1,2] [1]*University of Texas Health San Antonio, Department of Cellular and Integrative Physiology, San Antonio, TX, USA*; [2]*University of Texas Health San Antonio, Center for Biomedical Neuroscience, San Antonio, TX, USA*; [3]*Xiangya School of Medicine, Central South University, Changsha, Hunan, China.*

Among the most prominent acute neurological consequences of ethanol exposure are impaired motor coordination (ataxia) as well as impaired attention and memory. Noradrenergic signaling, originated by locus coeruleus neurons, plays important roles in attentional shifts, optimization of behavior-relevant brain circuit activity, and in gating synaptic plasticity. Ethanol can reduce locus coeruleus activity; however, the functional consequences at the cellular and circuit level are not well understood. To overcome this obstacle, we employed a linear treadmill in combination with in vivo two-photon calcium imaging on mice expressing the genetically encoded calcium indicator GCaMP3 in cerebellar Bergmann glia. Our previous experiments have revealed that locomotion leads to norepinephrine-dependent calcium activation of Bergmann glia in the cerebellum. Here, we found that locomotion-induced calcium elevations in Bergmann glia are reversibly and dose-dependently inhibited by ethanol exposure. Acutely enhancing norepinephrine release was sufficient to completely reverse this effect of ethanol suggesting that suppression of norepinephrine release might be the underlying mechanism by which ethanol inhibits behavioral state-dependent astroglia calcium activation. We investigated the consequences of ethanol impairment of astroglia calcium activation for calcium dynamics in local neurons. We recorded locomotion-induced calcium elevations in Purkinje cells, the principal neurons of the cerebellar cortex, using transgenic mice expressing the genetically encoded calcium indicator GCaMP6f. Locomotion induced a slow calcium elevation in Purkinje cells with an onset ~2 s following the onset of locomotion and lasting for 5-10 s, similar to corresponding calcium elevations in Bergmann glia. Prolonged Purkinje cell calcium elevations were inhibited by ethanol exposure and could be recovered by acutely enhancing norepinephrine release, suggesting the recruitment of a similar signaling pathway, or a hierarchical relationship between Bergmann glia and Purkinje cells. Our findings provide a cellular and circuit mechanism that may contribute to impairments of attention by acute ethanol exposure.

Additional Abstracts

48. Optimizing the two-photon excitation for Swept Confocally Aligned Planar Excitation (SCAPE) Microscopy. HANG YU, P. THILANKA GALWADUGE, VENKATAKAUSHIK VOLETI, ELIZABETH M.C. HILLMAN, *Laboratory for Functional Optical Imaging, Department of Biomedical Engineering, Columbia University, New York, NY, U.S.A.*

Two-photon laser scanning microscopy (2PLSM) has become one of the most widely used tools in neuroscience research. Owing to the reduced scattering of biological tissues at near-infrared wavelengths, 2PLSM has allowed researchers to monitor brain activity in vivo at cellular resolution to several hundred microns in depth. However, conventional 2PLSM relies on raster scanning of a focused point to form an image. Prohibitively high pixel rates are thus needed for 3D imaging at high speeds, limiting the ability of 2PLSM to image the dynamics of neural activity over large areas or volumes in the mammalian brain. We recently developed Swept Confocally Aligned Planar Excitation (SCAPE) microscopy (Bouchard et al. Nat. Photon. 9.2 (2015): 113-119.), a single-photon light-sheet microscopy technique that is capable of imaging living tissues at over 10 volumes per second. SCAPE microscopy uses a single objective lens for both excitation and fluorescence detection, illuminating the sample with an oblique light sheet whose position is imaged back to an obliquely co-aligned fast sCMOS camera. SCAPE's scan-descan approach enables rapid movement of the oblique illumination plane through the sample without physical translation of the sample or objective lens while maintaining the camera's focus on the sweeping light sheet. Using a single-photon 488 nm laser SCAPE microscopy's penetration depth is limited to 300-400 microns, dependent on the samples' absorption and scattering properties at visible wavelengths. However, extending SCAPE to utilize two-photon excitation could enable fast, volumetric imaging deep into the mammalian brain. Implementing two-photon SCAPE microscopy requires careful consideration of two-photon excitation efficiency as well as tissue damage thresholds. Two-photon excited fluorescence is proportional to the square of the incident power, such that simply expanding a standard two-photon point source to a light sheet would not permit high speed volumetric imaging. However, in our initial work we have demonstrated several key advantages of the SCAPE approach, including the efficiency of extending illumination axially rather than laterally, as well as the benefits of parallelized detection which permits much longer per-pixel integration times than point scanning, combined with the ability to utilize much lower repetition rate lasers. In recent tests, we have achieved >10 volume per second imaging in mouse brain. Our latest progress will be presented.