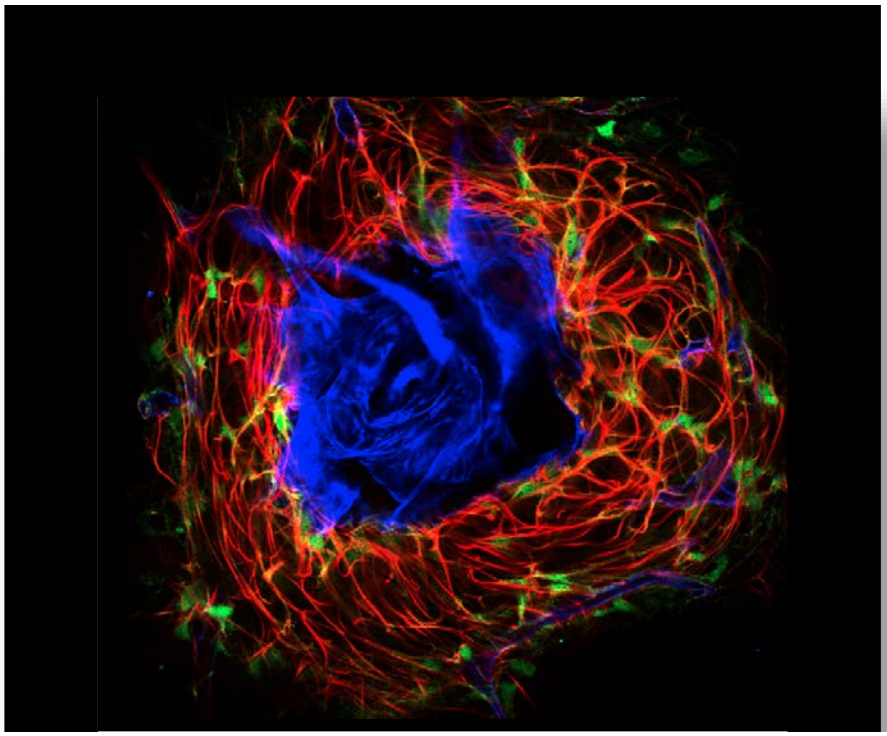

Neuroscience Research Institute

Co-Directors,
Stuart Feinstein &
Kenneth S. Kosik

MISSION STATEMENT

The mission of the Neuroscience Research Institute is to foster knowledge and understanding of the nervous system by serving as a center for scientific research breakthroughs. The NRI is a group of investigators whose collective goal is to create an intellectual atmosphere conducive to exploration at the frontiers of human knowledge where disciplinary boundaries disappear. Investigators in the NRI recognize that the interests of neuroscience extend broadly from repair and prevention of human disease to the principles that underlie the earliest nervous systems, from the human mind to the single molecular building blocks of the brain.

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Director's Statement

by Kenneth S. Kosik & Stuart Feinstein

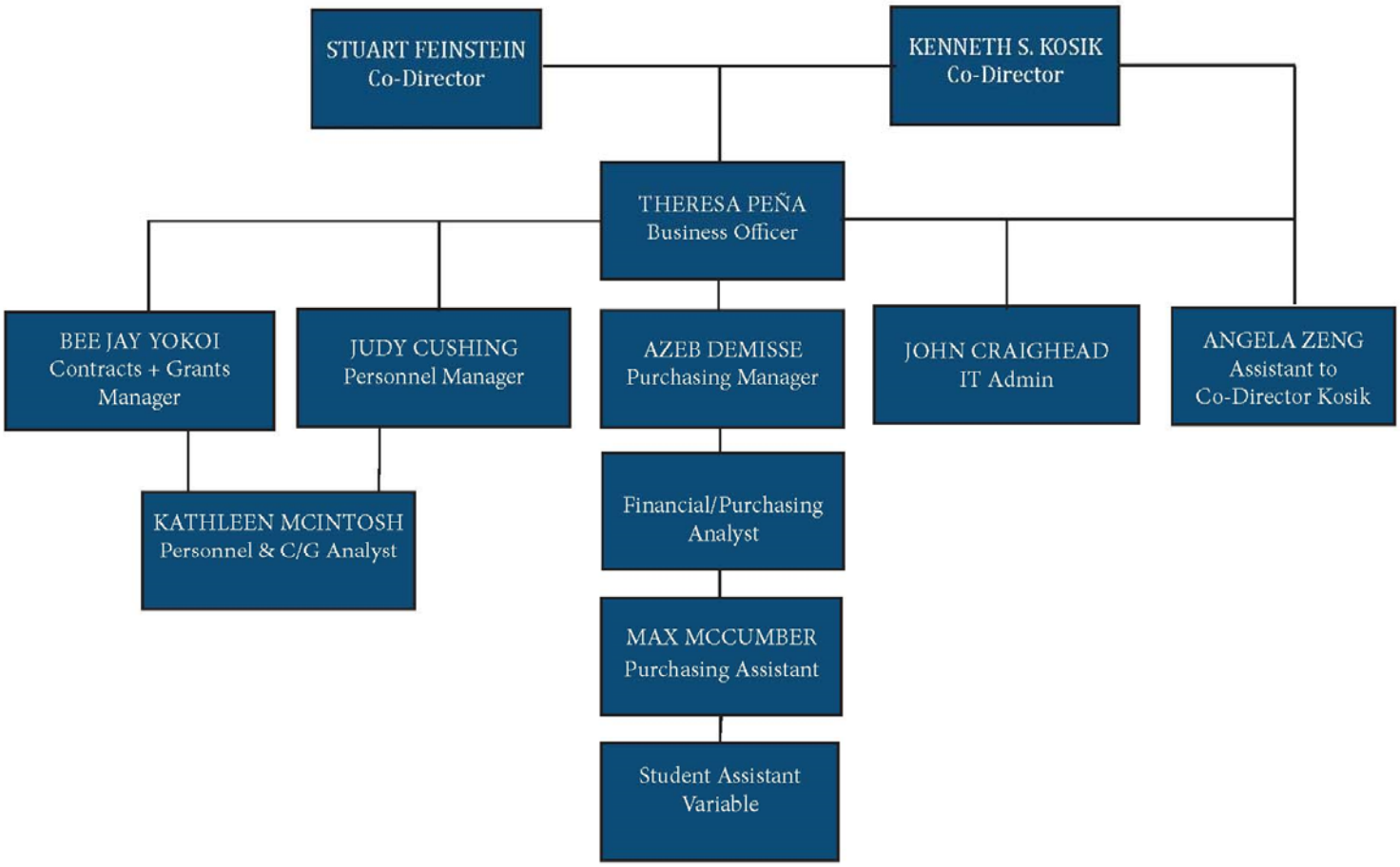
Neuroscience is increasingly emerging as a scientific priority at the national level, both in government agencies and on university campuses. The Neuroscience Research Institute (NRI) is the hub of neuroscience research at UCSB. Our mission is: (i) to serve the research interests of the campus in all facets of neuroscience; (ii) to promote collaborative, inter-disciplinary neuroscience research at UCSB; (iii) to train the next generation of neuroscientists. Our work includes both basic science and translational studies directed toward developing therapeutic strategies for the many neurological maladies afflicting society.

The NRI was instrumental in establishing the UCSB Brain Initiative in 2015-16, a campus-wide effort to increase the presence and breadth of neuroscience at UCSB. In collaboration with academic departments, NRI and the Initiative have assisted with the recruitment and successful hiring of leading neuroscience faculty including Drs. Matthieu Louis, Michael Goard, Emily Jacobs and Julie Simpson. Furthermore, another recruitment is presently underway for a Full Professor of Neuro-engineering within the College of Engineering. In addition to contributing to the recruitment of new faculty, NRI and the Initiative are working with existing faculty from many disciplines to identify novel relationships between their own ongoing research interests and neuroscience. New brain-related research proposals have been developed and submitted across MCDB, Physics, Electrical and Chemical Engineering, Computer Science, Mechanical Engineering, Psychology and Brain Sciences, Bioengineering, and EEMB, including successful proposals funded NSF and DARPA. New research partnerships have been established with Lawrence Berkeley National Labs, Huntington Memorial Research Institute, and the University of North Carolina, Chapel Hill, and proposals are in development for a Neurotechnology hub at UCSB as well as a Center for Adaptive Network Dynamics. New outreach and philanthropic initiatives are underway with the Office of Development. Finally, we continue to contribute to the neuroscience environment with many neuroscience seminars during the year, including our outstanding series of lectures supported by Gus Gurley, which has brought many distinguished neuroscientists to the campus over the years. We plan to complement these formal seminars with a new series of informal coffee hours and community events.

The NRI continues to promote research on campus through its Core Facilities in Light and Electron Microscopy and Stem Cells. These facilities are essential for the research of numerous investigators across campus in over a dozen departments/units in the Life Sciences, Physics, Chemistry, Materials and Engineering. Two successful extramural grants to the NIH and NSF totaling ~$1.2M will enable us to purchase two new, state-of-the-art light microscopes in 2017, keeping our work right at the cutting-edge. NRI also promotes neuroscience research on campus with its outstanding administrative support. Our administration and faculty work together in an environment of professionalism, excellence and cooperation that greatly facilitates the many administrative components of grant management. Under the superb chairmanship of Denise Montell, the NRI advisory committee has provided sage counsel to the NRI Co-Directors.

As we move forward to the coming year, the NRI will increasingly serve as a scientific and administrative home to ongoing neuroscience research as well as for efforts to broaden, enhance and better integrate neuroscience research across the campus.

Organizational Chart



Center and Facility Reports

UCSB Center for Stem Cell Biology and Engineering

Recent Activity

www.stemcell.ucsb.edu

Co-Directors and Holders of the Ruth Garland Endowed Chair:
James A. Thomson, Hyongsok "Tom" Soh

Executive Directors: Dennis O. Clegg, Peter J. Coffey

9-21-15

Research Highlights:

Significant progress was made in developing stem cell therapies for ocular disease, in understanding molecular mechanisms of stem cells, and in devising novel biotechnologies. News items are posted on our web site. Additional details are given below:

Research Progress and Achievements

I. Age-related Macular Degeneration. UC Santa Barbara does not have a medical school, and one major highlight of this university's research (which is perhaps unexpected), funded in part by the Garland Initiative, is the effort to develop cellular therapies for age-related macular degeneration (AMD). AMD is the leading cause of blindness in the USA, and for most patients, there are no treatment options. We have made significant progress in the last year in four areas:

A) The California Project to Cure Blindness. This is a collaborative effort between UC Santa Barbara, USC, Caltech, City of Hope and University College London, with funding from the Garland Initiative, the California Institute for Regenerative Medicine, the Breaux Foundation, the US Army, and other sources. This group includes Dr. Clegg as Co-PI and Dr. Coffey as investigator, with Dr. Thomson on the Scientific Advisory Board. We initiated a clinical trial called "CPCB-RPE1 implant - Human Embryonic Stem Cell-Derived Retinal Pigment Epithelial (RPE) Cells Seeded on a Polymeric Substrate; A Phase I/IIa Open Label Single Center Study to Assess the Safety and Tolerability of CPCB-RPE1 in Patients with Age Related Macular Degeneration (AMD)". The first patient was treated at USC, and additional patients are currently scheduled for surgery. Results thus far are promising. We plan to release clinical data after three patients have been treated.

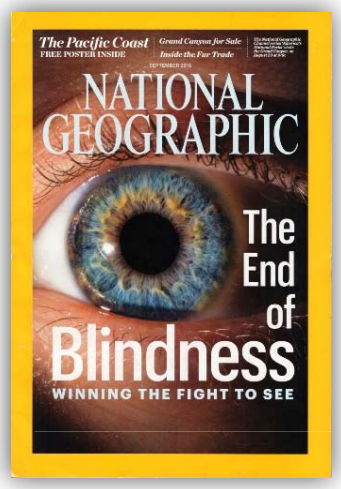


Figure 1 This work was featured in a cover story of the September issue of National Geographic.

B) The London Project to Cure Blindness. This project, initiated by Dr. Coffey, is targeting patients with the "wet" form of AMD who do not respond to drugs. This therapy also employs a human embryonic stem cell-derived RPE cells on a scaffold, but uses a different cell line, a different scaffold material, and targets a different patient population. The first two patients were operated in August and October of 2015. Both patients suffered from AMD and experienced a rapid decline in vision as a result of a severe bleeding into the sub-retinal space. The replacement RPE cells were implanted into the macular region on an artificial membrane. Post-surgery the cells remain viable, well placed and the retina is flat overlying the transplanted cells. Visual outcome is evident and has continued to improve over a 12month period: the first patient has gained a 6 line and the second patient a 5 line visual improvement.

C) Combining Photoreceptors with RPE. The Clegg and Thomson labs are part of a team developing ocular therapies using induced pluripotent stem (iPS) cells, funded by the Garland Initiative in combination with a Wynn-Gund Translational Research Acceleration Program Grant from the Foundation Fighting Blindness. In the past year, we were able to combine IPSC-derived photoreceptors with RPE cells on a scaffold and initiate proof of concept studies in a rat model. This combination of cells holds promise for AMD and other retinal diseases.

D) AMD and the Wound Response. Prominent loss of vision is only associated with late or advanced AMD. The transition from early to advanced AMD has features consistent with the onset of a wound response resulting from an underlying degenerative disease and chronic inflammation. Another area of research in Dr. Coffey's lab is directed at furthering our understanding of the molecular basis of RPE wound response, its likely impact on RPE function, and possible role in AMD. Using a systems biology / transcriptome approach, we identified changes in expression in a number of genes encoding products that play a role in wound response or the cell cycle. Finally, we have been investigating the possible role for wound responses in AMD by comparing the changes in gene expression in this cell culture model to previously reported AMD-associated gene expression changes in human pathological samples (Radeke et al., 2015).

II. Diabetic retinopathy. Diabetic retinopathy (DR) is the leading cause of vision loss and blindness in the working-age population, affecting 75% of diabetic patients within 15 years of diagnosis (Cheung, Mitchell et al. 2010). Unfortunately, patients with DR are faced with limited effective treatment options; current DR therapeutic options depend on highly invasive surgery performed only at advanced stages of the disease and are ineffective in restoring visual acuity. The earliest and most specific sign of DR is a reduction in the number of pericytes in the retinal capillary. Pericytes are contractile cells that support vascular function throughout the body. Pericyte loss leads to vascular aberration and impairment of the inner blood-retina barrier resulting in macular edema, the leading cause of vision loss in diabetic patients.

We continue to work with *Arvicanthis Nilothicus*, also known as the African grass rat or the Nile rat as a model for DR. Nile Rats have a propensity to develop type 2 diabetes when fed regular laboratory rodent pellets and can live with the disease for one to two years without insulin treatment or other medical intervention. However, a high fiber diet protects them from developing diabetes (Noda, Melhorn et. al, 2010). Vascular abnormalities consistent with known features of diabetic retinopathy (Noda, Nakao et al. 2014) have been reported, but when DR begins and how it proceeds is not yet clear.

In order to understand when DR develops, we have been monitoring the progression of type 2 diabetes effects on the retinal vascular network, in vivo, via fundus fluorescein angiography. Using these images, we have been exploring ways to quantitatively measure the development of DR by measuring vessel tortuosity, branching angle coefficients, and central artery to central vein ratios.

Retinal staining via immunohistochemistry was performed at various time points to quantify the dropout of distinct retinal cell types, as this indicates development of DR. This technique was developed in collaboration with the Reese lab at UCSB.

Furthermore, due to the need to identify the time when pericyte dropout occurs and the lack of well-staining pericyte antibodies, we have begun conducting trypsin digests on retinas. This is a procedure that involves washing away all of the neuronal cells leaving only the vascular network intact. This permits the quantification of retinal pericytes, as well as qualitative markers of the disease, such as acellular capillaries.

In addition to this, we are in the process of evaluating a select number of drugs to evaluate whether they are an effective treatment for diabetes in this model and beyond. Candidate drugs were identified by computational drug repositioning methods using data mined from electronic health records (Kuang, Thomson et al, 2016).

Lastly, we performed RNA-sequencing on over 35 different organs and tissues, including the retina, as a preliminary step in assembling a Nile rat transcriptome. Fosmid sequencing for future genome assembly has also been completed using institutional funding. Together, these studies will provide the data needed to establish the temporal framework of DR in the Nile Rat, and we will continue doing so in the coming year.

III. Bioengineering real-time biosensors and feedback- control.

Dr. Soh is now at Stanford University, with his appointment split between Medicine and Engineering. However, Dr. Soh has retained his laboratory at UCSB, and continues to collaborate within the Stem Cell Center as part of the Garland Initiative at UC Santa Barbara. This move has facilitated interactions between the Garland Initiative and Stanford, with its world class Medical School.

The main achievement in the past year has been the development of Xeno Nucleic acid Aptamers (XNAs) that have programmable chemical functionality to target virtually any biomolecule including recalcitrant proteins and small molecules. To achieve this, we developed non-natural nucleic acids that have "click-chemistry handles" such that any desired chemical functional groups can be conjugated to the aptamer. Then we screen the XNA library using the "particle display" strategy previously developed our group that can screen as many as 10^8 individual XNA's in a high-throughput manner. As proof of concept, we have XNA molecules for model proteins with exceptional affinity and specificity (Niu et al. in preparation 2016). In the upcoming year, we will use this platform to generate XNA reagents for important protein targets of diabetic retinopathy and age-related macular degeneration (such as the targets identified by Dr. Coffey in D) above) in collaboration with the efforts of Clegg, Coffey and Thomson.

IV. Retinitis Pigmentosa (RP)

Steve Fisher's group has been instrumental in obtaining preclinical data in support of a cellular therapy for RP being developed at UC Irvine. Recently, the FDA cleared their IND application, and a cohort of patients were treated by injecting retinal progenitor cells into the eye. (see UCSB press release).

Efforts continue to understand the molecular basis of RP, using iPS cells to model the disease in cultured retinal cells. Dr. Coffey's lab has studied the role of the CEP290 gene, and recently published a possible avenue towards treatment in the journal Cell Stem Cell. Drs. Clegg and Thomson are collaborating with Dr. Eric Pierce at Harvard to investigate mutations in the PRPF genes, which are involved in RNA splicing.

V. Education and Outreach

An important part of the Stem Cell Center is education and outreach to the public. To that end, we have developed two websites that describes our research: stemcell.ucsb.edu and http://garland.stemcell.ucsb.edu. We have also continued a series of seminars where outside experts can come and interact with researchers at UCSB (see

http://garland.stemcell.ucsb.edu.) Our labs were opened to interns from high schools and undergraduate institutions over the summer as part of the CIRM Research Mentorship Program and the Institute for Creative Biotechnology Program (https://www.youtube.com/watch?v=QSeVhUlsg5k). In addition, faculty traveled to outside universities and presented our research. One highlight was the annual meeting of the International Society for Cell Therapy in Memphis, TN, where Drs. Clegg and Coffey were keynote speakers.

In the past year, we worked with Americans for Cures (founded by Robert Klein) to develop an excellent white board animation about the California Project. Please see:

http://americansforcures.org/amd-age-related-macular-degeneration

Over the past year, in addition to many seminars at meetings and universities, the Stem Cell Center faculty was also engaged in outreach via seminars to general audiences at various public venues. These included: The Vista Del Monte Retirement Community, Santa Barbara, CA; The American Association of Blood Banks, Anaheim, CA.; The UC Santa Barbara Board of Trustees Meeting; The Envision Pre-Optometry Club, UC Santa Barbara, Santa Barbara, CA; The Biotechnology Mixer, California State University Channel Islands, Camarillo, CA; MCDB 12 Career Lecture, UC Santa Barbara, Santa Barbara, CA; Cottage Health / UCSB Collaborative Symposium, Santa Barbara, CA; Maravilla Retirement Community, Santa Barbara, CA; Oxnard College Interns, Santa Barbara, CA; American Association of Blood Banking, Webinar Regenerative Medicine: Ocular Therapy; UC Santa Barbara Summer Start Program, Santa Barbara, CA; Federal Drug Administration, Bethesda, MD.

The presentation at the FDA by Dr. Clegg was especially important as the agency considers new regulations for stem cell therapies. This workshop was part of a meeting where the public was invited to comment on FDA regulations regarding stem cell research.

In addition, our publications have received attention in the press (see news items https://stemcell.ucsb.edu).

VI. Center Facilities and Recent Faculty Recruitment:

- The Department of Chemical Engineering has hired Dr. Sid Dey, a stem cell bioengineer from The University of California, Berkeley, who will be a member of the Center and will utilize the Center Core Facility.
- Core Facility –The shared core facility – the Laboratory for Stem Cell Biology and Engineering, which has been operating since 2005, continues to thrive, with users from on campus and off campus. We transitioned to a new mechanism for funding with donations and recharge income.

NRI-MCDB Microscopy Facility

The NRI-MCDB Microscopy Facility, founded in 1990, is jointly maintained by the Neuroscience Research Institute and the Department of Molecular, Cellular and Developmental Biology at the University of California, Santa Barbara. The Facility's mission is to promote and facilitate microscopy-based research. To achieve this mission the Facility houses state-of the art instruments, supports expert full-time support staff, hosts outreach events and provides both individual and workshop-based training in microscopy.

The Facility is the primary light microscopy core on campus supporting researchers in more than 13 department/units including the Life Sciences, Physics, Chemistry, Materials and Engineering. The Facility has 102 registered Principal Investigators with use by 50 PIs this past year. In the past year, the Facility supported 138 users and 2352 reservations. Those reservations count for approximately 5000 hours of use for $110,000 of recharge income. The Facility users are asked to acknowledge the Facility in their publications and report new publications supported by the Facility. A list of the research publications that have been reported to the Facility in 2015-2016 conclude this report.
This centrally located Facility is based within the Neuroscience Research Institute, in the Biological Sciences II building. Presently, the Facility maintains multiple sophisticated instruments including a JEOL JEM-1230 transmission electron microscope, an Olympus Fluoview 1000 Spectral Confocal Laser Scanning Microscope, an Olympus Fluoview 1000 Multiphoton Laser Scanning Microscope and an Olympus Spinning Disk Confocal. The Facility also hosts five Olympus compound microscopes configured with transmitted and fluorescent light-paths as well as a stereomicroscope configured with transmitted and reflected light. These microscopes are further equipped with research grade digital cameras and a high-end computer workstation for image acquisition, processing, and analysis. The confocal and multiphoton microscopes are equipped with time-lapse software controls for automated long-term imaging and the Fluoview 1000 systems are equipped with a motorized X, Y stages for automated sampling of multiple locations. It also provides an Imaris 3D image processing and analysis workstation for the Facility users.

All but one of the instruments get significant use, and recharge fees manage to cover the service contracts overall. The Olympus Fluoview 1000 Spectral Confocal microscope was by far the biggest income source at $52,800 and nearly 1700 hours of use. The Multiphoton microscope was used very little with only 180 hours of use and $5,700 of recharge fees. That system has two service contracts: one for the MaiTai infrared laser and the other for the Olympus microscope itself. The two service contracts total to $30,535 making the multiphoton system a big drain on the facility. Those current service contracts are in effect until 3/1/2017, and closer to that time we will decide if those contracts should be renewed.
There are new instruments and technology coming to the Facility. This year Dr. Bill Smith was awarded a NIH shared instrumentation grant for a lightsheet microscope. We've chosen a Zeiss Lightsheet Z.1 which will be arriving in a few months. The lightsheet microscope generates huge data sets so along with the microscope we are getting a ~300 TB capacity data server, a new high-end analysis computer with Imaris, and 10 gigabit per second data ports added to the Facility.

We are moving to a new online scheduling system. The current system was set up by UC San Diego. While they continue to run the server for our Facility, they no longer use that system and no longer have the ability to fix it if something goes down. The new system, iLabSolutions, is a modern commercial system that integrates with GUS which will eliminate employee-hours manually transferring billing charges from the scheduling system to GUS. There is a yearly subscription fee for iLabSolutions (currently $1600) which will guarantee technical support if any issues arise.
Replacing Dr. Mary Raven as facility director in March 2016 is Dr. Benjamin Lopez. Ben has experience in doctoral and postdoctoral research involving microscope instrument design, imaging, and image analysis. Ben is assisted by Dr. Geoff Lewis who oversees transmission electron microscopy. Both Ben and Geoff have published numerous papers employing conventional, TIRF, optical trapping, transmission electron microscopy and confocal microscopy. Drs. Lopez and Lewis provide training on a daily basis and regularly meet with individuals to provide advice and to address additional microscopy needs.

Other University contributions

UC Work Group for Adaptive Optics in Biological Imaging
The UC Work Group for Adaptive Optics in Biological Imaging headed by Joel Kubby at UCSC was selected for support by the Multicampus Research Program. Dr. Ben Lopez is serving as the UCSB campus lead.

MCDB126BL: Basic Pharmacology
The main microscopy facility room 5173 and widefield epifluorescence microscopes were used in the basic pharmacology lab for 2 weeks.

MCDB133L: Immunobiology
The main microscopy facility room 5173 and widefield epifluorescence microscopes were used for one day.

MCDB161L: Research Immersion in Molecular Biosciences
The main microscopy facility room 5173 and widefield epifluorescence microscopes were used for three days.

Public Service and K-12 Outreach

The NRI-MCDB Microscopy Facility participates in campus-wide events as well as undergraduate and graduate student tours and orientations. In 2015 the Facility participated in Parent and Family Weekend and the All Gaucho Reunion. The Facility also hosted tours for the Condor Techs Summer Internship, and Brooks Institute scientific photography students.

Facility Supported Publications 9/2015-9/2016

2016

Reinig MR, Novack SW, Tao X, Ermini F, Bentolila LA, Roberts DG, MacKenzie-Graham A, Godshalk SE, Raven MA, Kubby J. 2016. **Adaptive optics microscopy enhances image quality in deep layers of CLARITY processed brains of YFP-H mice.** SPIE, Clinical and Translational Neurophotonics; Neural Imaging and Sensing; and Optogenetics and Optical Manipulation. 9690

Do TD, de Almeida NEC, LaPointe NE, Chamas A, Feinstein SC, Bowers MT. 2016. **Amino Acid Metaclusters: Implications of Growth Trends on Peptide Self-Assembly and Structure.** Anal Chem. 88(1):868-76.

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Mortimer M, Petersen EJ, Buchholz BA, Orias E, Holden PA. 2016. **Bioaccumulation of Multiwall Carbon Nanotubes in Tetrahymena thermophila by Direct Feeding or Trophic Transfer.** Environmental Science & Technology. 50:8876-8885.

Camacho KM, Menegatti S, Vogus DR, Pusuluri A, Fuchs Z, Jarvis M, Zakrewsky M, Evans MA, Chen R, Mitragotri S. 2016. **DAFODIL: A novel liposome-encapsulated synergistic combination of doxorubicin and 5FU for low dose chemotherapy.** Journal of Controlled Release. 229:154-162.

Benbow SJ, Cook BM, Reifert J, Wozniak KM, Slusher BS, Littlefield BA, Wilson L, Jordan MAnn, Feinstein SC. 2016. **Effects of Paclitaxel and Eribulin in Mouse Sciatic Nerve: A Microtubule-Based Rationale for the Differential Induction of Chemotherapy-Induced Peripheral Neuropathy.** Neurotoxicity Research. 29:299–313.

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Cai D, Dai W, Prasad M, Luo J, Gov NS, Montell DJ. 2016. **Modeling and analysis of collective cell migration in an in vivo three-dimensional environment.**Proceedings of the National Academy of Sciences.

de Almeida NEC, Do TD, Tro M, LaPointe NE, Feinstein SC, Shea J-E, Bowers MT. 2016. **Opposing Effects of Cucurbit[7]uril and 1,2,3,4,6-Penta-O-galloyl-β-d-glucopyranose on Amyloid β25-35 Assembly.** ACS Chem Neurosci. 7(2):218-26.

Rani N, Nowakowski TJ, Zhou H, Godshalk SE, Lisi V, Kriegstein AR, Kosik KS. 2016. **A Primate lncRNA Mediates Notch Signaling during Neuronal Development by Sequestering miRNA.**

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Clevenger TN, Hinman CR, Rubin RKAshley, Smither K, Burke DJ, Hawker CJ, Messina D, Van Epps D, Clegg DO. 2016. **Vitronectin-Based, Biomimetic Encapsulating Hydrogel Scaffolds Support Adipogenesis of Adipose Stem Cells.** Tissue Engineering Part A. 22(7-8)

2015

Diaz HMorales, Mejares E, Newman-Smith E, Smith WC. 2015. **ACAM, a novel member of the neural IgCAM family, mediates anterior neural tube closure in a primitive chordate.** Developmental Biology.

Pallaoro A, Braun GB, Moskovits M. 2015. **Biotags Based on Surface-Enhanced Raman Can Be as Bright as Fluorescence Tags.** Nano Letters. 15(10)

Yu D, Feinstein SC, Valentine MT. 2015. **Effects of wild type tau and disease-linked tau mutations on microtubule organization and intracellular trafficking in COS-7 cells.** Journal of Biomechanics.

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Eschmann NA, Do TD, LaPointe NE, Shea J-E, Feinstein SC, Bowers MT, Han S. 2015. **Tau Aggregation Propensity Engrained in Its Solution State.** The Journal of Physical Chemistry B. 119(45)

Ganguly P, Do TD, Larini L, LaPointe NE, Sercel AJ, Shade MF, Feinstein SC, Bowers MT, Shea J-E. 2015. **Tau Assembly: The Dominant Role of PHF6 (VQIVYK) in Microtubule Binding Region Repeat R3.** The Journal of Physical Chemistry B. 119(13)

Principal Investigator Reports

FISHER, STEVEN

"Photoreceptor disk membrane morphogenesis"

1 R01 EY024667-01

During the past year we have been actively pursuing research on the above NIH grant. This work is done in collaboration with Dr. David Williams at the Jules Stein Eye Institute, UCLA. We completed the first specific aim of the project and published those results (see below). We are now pursuing the 2nd aim of using electron microscope tomography to produce 3D reconstructions of photoreceptor outer segments in mice that carry a mutation known to cause retinitis pigmentosa in humans. We are also using the same technology to study disk morphogenesis in cone photoreceptors, the most important photoreceptors for human vision.

Publications:

Volland S, Hughes LC, Kong C, Burgess BL, Linberg KA, Luna G, Zhou ZH, Fisher SK,. Williams DS . 2015. Three-dimensional organization of nascent rod outer segment disk membranes. Proc Natl Acad Sci U S A. 112: 14870-5 PMID: 26578801

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Sajdak B, Yusufu SN, Langlo CS, Luna G, Fisher SK, Merriman DK, Dubra A. (2015). Noninvasive imaging of the thirteen-lined ground squirrel photoreceptor mosiac. Vis Neurosci. 33:E003. doi: 10.1017/S0952523815000346. PMID: 26923645.

Mandal N, Lewis GP, Fisher SK, Heegaard S, Prause JU, la Cour M, Vorum H, Honore B. (2015). Proteomic analysis of the vitreous following experimental retinal detachment in rabbits. J Ophthalmol. doi: 10.1155/2015/583040. PMID: 26664739.

LEWIS, GEOFFREY

1) GP Lewis (PI) and SK Fisher: CIRM Sub-award, project title: "Retinal progenitor cells for the treatment of retinitis pigmentosa".

Over the last year, in collaboration with Henry Klassen at UCI, we completed the pre-clinical work required by the FDA for a CIRM funded project using human retinal progenitor cells for the treatment of retinitis pigmentosa, and were subsequently approved to begin treating patients. At the NRI we demonstrated that the progenitor cells slowed the loss of photoreceptors without causing any adverse effects in the eyes of the RCS rat, an animal model for this disease. The studies, helped lead to clinical trials of this treatment strategy in patients with retinitis pigmentosa beginning in August of 2015. To date 28 patients have been treated, and many are reporting improved vision. Since then, we have continued to explore the use of the retinal progenitor cells at the NRI, using other disease models including glaucoma, retinopathy of prematurity, and diabetic retinopathy. The September 2016 issue of National Geographic describes our treatment strategy.

2) GP Lewis (PI) and SK Fisher: Grant from Galecto Biotech, Copenhagen, Denmark, project title: "An efficacy study of the anti-fibrotic potential of 2 Galectin-3 inhibitors in a rabbit model of proliferative vitreoretinopathy (PVR)."

In this project we tested the ability of several anti-fibrotic compounds developed at Galecto Biotech to reduce glial scarring in the retina after retinal detachment. Both compounds were remarkably effective and the company is now beginning the process to get approval to use the drugs in patients. The project was completed in January of 2016 at the NRI.

MA, ZACH

CRCC award: Title "A novel role of histone methyltransferase subunits in cytokinesis"

The final step of cytokinesis in animal cells is abscission, which requires microtubule disassembly at a specific midbody region called the secondary ingression. However, the mechanisms leading to microtubule disassembly are not well understood. We have show that WDR5, a core subunit of histone H3 lysine 4 methytransferase complexes, is targeted to the midbody in the absence of chromatin and promotes formation of secondary ingressions by facilitating microtubule disassembly.

Cottage award: Title "Histone methyltransferase and cytokinesis"

Following the above finding that WDR5 promotes secondary ingression formation and abscission, we set forth to identify proteins interacting with WDR5 during cytokinesis and discovered two novel WDR5-interacting partners, GEFH1(a microtubule-dependent activator of Rho GTPase) and KIF2A(a microtubule depolymerizing protein). We are currently investigating the biological functions of these interactions.

Public Services

Our findings about the role of histone methyltransferase subunits in cytokinesis have been featured in UCSB press release in 2015.

Our lab has been mentoring two students from Adolfo Camarillo High School since the summer of 2016

JORDAN, MARY, Adjunct Professor And Research Biologist, Recalled

In collaboration with P.I. Professor Feinstein and CoPI Professor Wilson we have obtained a new grant from Genentech in the amount of **$ 326,264** to examine the mechanisms of action of a novel cancer drug called Auristatin. **Title: Mechanisms of Action of MMAE as a Free Drug and an Antibody Drug Conjugate**

In addition we have published 3 new papers examining the induction of peripheral neuropathy by microtubule-targetting drugs and the role of alterations in expression of beta tubulin isotypes on the actions of microtubule-targeted drugs. These drug actions are important in determining the efficacy of these anti-cancer drugs and in designing novel cancer treatments. A fourth manuscript, supported by a grant from Sanofi, Inc. is in the process of submission for publication.

PUBLISHED:

Structural basis for induction of peripheral neuropathy by microtubule-targeting cancer drugs. Smith JA, Slusher BS, Wozniak KM, Farah MH, Smiyun G, Wilson L, Feinstein S, Jordan MA. Cancer Res. 2016 Aug 3. pii: canres.3116.2015. Supported by a grant from Eisai, Inc.

Effects of Paclitaxel and Eribulin in Mouse Sciatic Nerve: A Microtubule-Based Rationale for the Differential Induction of Chemotherapy-Induced Peripheral Neuropathy. Benbow SJ, Cook BM, Reifert J, Wozniak KM, Slusher BS, Littlefield BA, Wilson L, Jordan MA, Feinstein SC. Neurotox Res. 2016 Feb;29(2):299-313. Supported by a grant from Eisai, Inc.

Effects of eribulin on microtubule binding and dynamic instability are strengthened in the absence of the βIII tubulin isotype. Wilson L, Lopus M, Miller HP, Azarenko O, Riffle S, Smith JA, Jordan MA. Biochemistry. 2015 Oct 27;54(42):6482-9. Supported by a grant from Eisai, Inc.

Nearly ready for submission for publication (Supported by the grant from Sanofi, Inc.): βIII-tubulin expression enhances efficacy of cabazitaxel as compared with docetaxel, Gregoriy Smiyun[12], Olga Azarenko[12], Herb Miller[1], Alex Rifkind[1], Leslie Wilson[1], and Mary Ann Jordan[1]

VANDENBERG, CAROL

"Neuronal Polarity"; Carol Vandenberg, principal investigator

Neurons are specialized for directional transfer of information, and require unique sets of membrane proteins in axonal and dendritic regions. The SNARE protein complex underlies intracellular membrane fusion events, but its role in neuronal polarity and selective protein targeting have been unclear. Our findings suggest an important role for the SNARE protein syntaxin 3 in maintaining neuronal polarity and in the critical task of selective trafficking of membrane proteins to axons.

Publication:

Soo Hoo, L., Banna, C. D., Radeke, C. M., Sharma, N., Albertolle, M. E., Low, S. H., Weimbs, T., and Vandenberg, C. A. (2016) The SNARE protein syntaxin 3 confers specificity for polarized axonal trafficking in neurons. PLOS ONE (in press) DOI: 10.1371/journal.pone.0163671

DOYLE, ADELE

DA01 15-16: Funds spent to get desk chairs for the lab, to accommodate more researchers joining the group. Remaining start up funds reserved while spending down on other expiring awards.

DA03 15-16: Microscopy recharges. Current projects in the group use fluorescence microscopy to monitor neural network growth in 3D gels and track neuron subtype specification during directed differentiation of mouse embryonic stem cells. We established a 3D culture protocol enabling survival and neuronal differentiation of mouse ES cells that permits longer extension of neurite clusters compared to 2D culture. We demonstrated current best practices for forebrain neuronal directed differentiation result in substantial heterogeneity, and are developing an immunofluorescence assessment panel to screen alternate methods for active synapse development and neurotransmitter identity.

1. Hosted 5 high school interns in intensive research experience during Summer 2016 as part of the UCSB RMP program.

2. Mentored a team project entry in the Siemens Competition in Math, Science & Technology
(leading science and mathematics research based competition for high school students in the United States)

Co-author on Nature Methods paper. Thomsen ER, Mich JK, Yao Z, Hodge RD, Doyle AM, Jang S, Shehata SI, Nelson AM,Shapovalova NV, Levi BP, Ramanathan S. Fixed single-cell transcriptomic characterization of human radial glial diversity. Nat Methods. 2016 Jan;13(1):87-93. doi: 10.1038/nmeth.3629. Epub 2015 Nov 16. PubMed PMID: 26524239; PubMed Central PMCID: PMC4869711.

Awarded one of 18 newly funded projects from the cross-disciplinary NSF Integrative Strategies for Understanding Neural and Cognitive Systems program, which supports bold efforts to go beyond single-discipline research efforts in order to advance brain science. The awards will contribute to NSF's significant investments in support of the BRAIN Initiative, a coordinated research effort that seeks to accelerate the development of new neurotechnologies. Each award provides a research team with up to $1 million over two to four years. Multidisciplinary team: Kimberly Turner (Lead PI), Megan Valentine (Co-PI) and Adele Doyle (Co-PI) of the University of California, Santa Barbara, µHammer: Impacting neuroscience one cell at a time. (Press announcement: http://www.infozine.com/news/stories/op/storiesView/sid/65221/)

(Note - one of the other 18 awards also went to a UCSB/NRI alum - Danielle Bassett of the University of Pennsylvania and Fabio Pasqualetti of the University of California, Riverside, A mechanistic model of cognitive control.)

KIPPIN, TOD

1) The Brain Booth at Hope Elementary School for their STEM Night (January 2016)

2) Brain Awareness Week programming for all K-6 classes at Hope Elementary School (Feb-March, 2016), consisting of 45-min presentations during the class room's regularly scheduled Science class.

Over the course of the fiscal year, my laboratory published a total of 5 scientific articles.

SZUMLINSKI, KAREN

1) The Brain Booth at Hope Elementary School for their STEM Night (January 2016)

2) The Brain Booth at Girls Inc. Goleta Valley for their STEM Festival (April 2016)

3) Brain Awareness Week programming for all K-6 classes at Hope Elementary School (Feb-March, 2016), consisting of 45-min presentations during the class room's regularly scheduled Science class

4) Brain Awareness Week programming for all K-6 classes at Santa Barbara Charter School (April 2016), consisting of 45-min presentations held in each classroom

5) Brain Awareness Week programming for all 7 Preschool classrooms at Orfalea Family Children's Center (March 2016), consisting of 45-min presentations in the center's multipurpose room

see podcast here: http://www.news.ucsb.edu/2016/016963/brain-lady

Over the course of the fiscal year, my laboratory published a total of 11 scientific articles and 1 commentary. Of these articles, 5 appear in journals with IF>6 (incl. J Neurosci, Biol Psychiat, Addiction Biol). I also have 1 additional revised report currently under review and another report under initial review). I have 1 book chapter in press.

Awards Administered

UNIVERSITY OF SOUTHERN CALIFORNIA
Dennis Clegg 8/1/2014-7/31/2017 $367,361
Phase 1 Safety Assessment of CPCB-RPE1, hESC-derived RPE Cell Coated Parylene Membrae Impants, in Patients with Advanced Dry Age Related Macular Degeneration

UCSB will develop a method for cryopreservation of CPCB-RPE1 – the final product being developed for treatment of age-related macular degeneration. The product consists of a mature monolayer of hESC-RPE grown on a vitronectin-coated parylene C membrane with ultrathin areas. Modern methods of cryopreservation and vitrification will be carried out, testing variety of parameters to optimize the method for maximal cell survival and function after thaw. We will explore various modifications of cell maturation protocol, cryopreservant, cryopreservation and vitrification methodology, transport, and protocols for thawing and recovery. Cells will be assayed after various times to determine shelf life. Assays of cells after thaw will include qPCR, ICC, ELISA measurement of growth factor secretion, and phagocytosis of photoreceptor outer segments. Cryopreseved CPCB-RPE1 will then tested in animal models of RPE dystrophy at USC to assess function in vivo.

UNIVERSITY OF WISCONSIN
Dennis Clegg 10/11/13-9/30/16 $255,000
Co-culture and analysis of neural retina and RPE derived from GMP super donor hiPSC lines
Generation of RPE cells from super donor iPS lines.

Development and analysis of planar scaffolds for iPS-RPE.
Development and analysis of PEG hydrogels for encapsulation of iPS-neural retinal cells.
Characterization of cell constructs in vitro and in vivo.

NRI RESEARCH FUNDS
Adele Doyle

The Doyle group studies two interfaces linking physical forces with intracellular decision making circuits: the developmental of electrochemical signaling in differentiating neurons and the acquisition of mechanosensitivity in the vascular system. Initial efforts in the lab are executed via collaborations with the Theogarajan (shared graduate student: Sarah Grundeen), Kosik, Hansma, and Campas (shared graduate student: Adam Lucio) laboratories. Standalone efforts by students and visiting scientists in the Doyle group are using developing novel computational and high throughput data analysis methods to identify and simulate the molecular regulatory circuits necessary for electrical and mechanical force sensing in cells. Computational predictions will be tested in vitro as the projects progress.

CIRM CREATIVITY MENTOR AWARD
Adele Doyle 6/1/2012-12/1/2015

During Summer, 2015, four CIRM interns participated in research in the Doyle Group. Their efforts collectively: identified a suitable protocol for freezing primary neurons, a notoriously finicky cell type for cryopreservation; identified molecules participating in a genetic regulatory switch between GABA and Glutamatergic neuron specification; and enhanced an existing bioinformatics pipeline in the lab enabling rapid Gene Ontology-based data analysis to include human and zebrafish data. These funds also provided computer workstations in the lab that are currently being used to further these research projects.

EISAI RESEARCH INSTITUTE
Stuart Feinstein 11/1/2014-10/31/2017 $630,772
Recovery from Chemotherapy-Induced Peripheral Neuropathy: Focus upon Neuronal Cell Biology and Biochemistry

The goal of the work described here is to acquire a detailed molecular characterization of key components of the recovery process from microtubule-targeted agent (MTA) induced CIPN. More specifically, we propose:

(i) to characterize the recovery from MTA-induced CIPN using molecular and morphological markers of neuronal cell death (i.e., apoptosis), inflammation and peripheral nerve regeneration;

(ii) to characterize the cellular recovery from MTA-induced CIPN with respect to microtubule biochemistry in neurons.

This work will integrate with work described in the accompanying proposal from Dr. Slusher and her colleagues at Johns Hopkins University. Together, we will develop an inter-disciplinary understanding of the differential abilities of animals to recover from MTA-induced CIPN, expanding upon and complementing our ongoing work examining the differential effects of different MTAs to induce CIPN.

EISAI RESEARCH INSTITUTE
Stuart Feinstein 2/1/2015-6/30/2015 $3,700
Chemotheraphy-Induced Peripheral Neuropathy Symposium

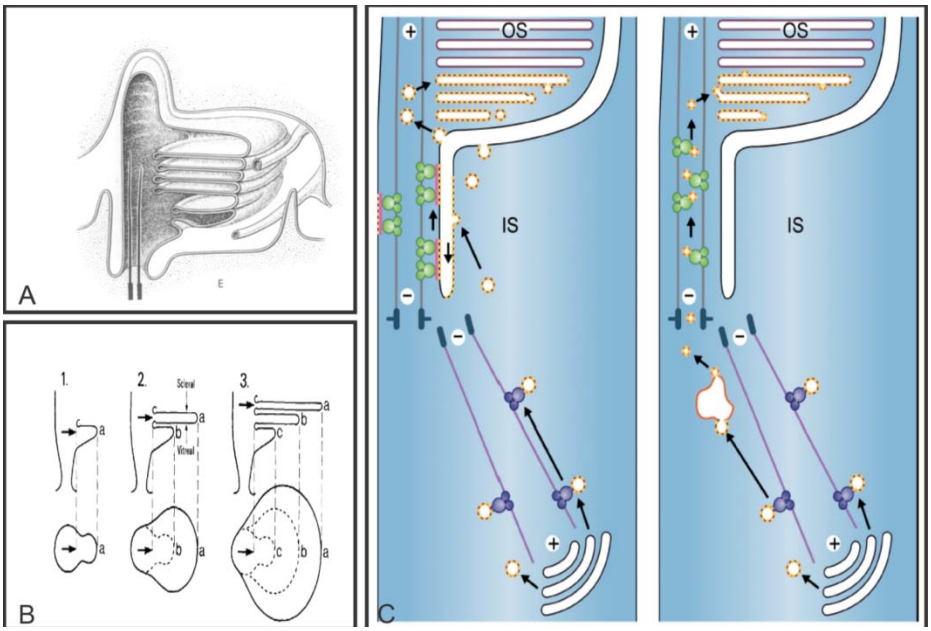
Our goal through this symposium is to foster dialog between basic and clinical researchers within the field of CIPN so that the understanding of mechanistic causes can ultimately translate to CIPN treatment and management in patients. Reciprocally, the information gained in the clinic will benefit basic research by informing the prioritization of endeavors. Therefore the target audience includes basic science researchers as well as clinicians and medical professionals.

GENETECH
Stuart Feinstein 5/2/2016-5/2/2017 $326,264

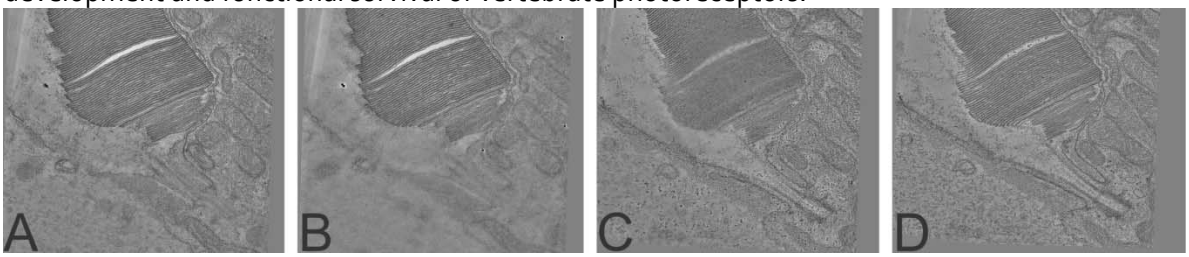
Microtubule targeting agents (MTAs) are valuable chemotherapeutic drugs for many types of cancer (Windebank and Grisold, 2008, Argyriou et al., 2011, Carlson and Olsen, 2011, Argyriou et al, 2012,). Mechanistically, the anti-tumor activity of MTAs stems from their ability to alter normal microtubule dynamics and/or the regulatory mechanisms controlling microtubule dynamics and microtubule-based transport, which can in turn lead to tumor cell death (Jordan and Wilson 2004, Agryriou et al., 2012, Field et al., 2014, Poruchynsky et al., 2015). Unfortunately, systemic delivery of MTAs also exposes non-target tissues to chemical assault that can cause significant adverse side effects. For example, lacking the protection conferred upon the central nervous system by the blood brain barrier, the peripheral nervous system is highly susceptible to deleterious MTA-induced effects. Among the most frequent and serious side effects of MTA treatment is chemotherapy-induced peripheral neuropathy (CIPN), which can manifest itself with symptoms ranging from numbness and tingling to hypersensitivity and severe neuropathic pain. CIPN symptoms generally exhibit a "stocking/glove" pattern beginning at the most distal extremities, such as fingertips and toes, and progresses proximally toward the trunk, suggesting that the longest axons are the most vulnerable (Windebank and Grisold, 2008, Carlson and Ocenan 2011, Argyriou et al. 2012). These symptoms can be sufficiently debilitating as to be treatment limiting, and in some cases, even life threatening. Indeed, the only known strategies to address CIPN are to either reduce drug dosage or cease treatment altogether, both of which undermine attempts to control a patient's cancer. Since MTAs are a major component of available clinical anti-cancer strategies, CIPN poses a major obstacle to successful clinical anti-cancer efforts.

NIH National Eye Institute
Steven Fisher 4/1/2015-3/31/2019 $384,375
Summary: NIH grant 1 R01-EY024667-01 "Photoreceptor Disk Membrane Morphogenesis"

The research will provide a fundamental mechanistic understanding of the way disk membranes of the photoreceptor outer segment are formed. It will apply novel 3-D image analyses to resolve nano-level structures of nascent disk membranes, and thus take the field of photoreceptor cell biology a major step forward. The research will include studies of mouse models retinal degeneration, specifically retinitis pigmentosa, cone-rod dystrophy, and macular degeneration, and thus will provide a basis for understanding the pathogenesis of these forms of blindness.



Drawings showing the proposed models of disk morphogenesis in vertebrate rod photoreceptors. The aim of this project is to determine at subcellular levels of resolution and in 3-dimensions the best-fit model for this critical event in the development and functional survival of vertebrate photoreceptors.



A figure showing data being collected for this project in collaboration with Dr. David Williams (co-investigator) at UCLA. The four panels show representative electron tomography images through a single rod photoreceptor outer segment. The images are from a "tilt series" of images taken from two sections, each 350 nm thick and then stitched together to form one continuous final tomogram. Each image (z-slice) in the tomogram is about 2.7 nm thick. The two dark parallel lines to the bottom right of C and D are the basal body that gives rise to the ciliary stalk that forms the light sensitive outer segment, the stack of parallel structures in the center of each panel. This data is used to create high-resolution 3D models of the outer segments at the critical point of new disk formation.

NIH- MENTAL HEALTH
Michael Goard 2/8/2016-1/31/2017 $256,536

Dissection of the neural circuitry of short-term memory in behaving mice
Short-term memory, the ability to hold information in mind over short timescales, is a fundamental cognitive process underlying an array of complex abilities. In contrast to long-term memory, which involves modification of synaptic connections, short-term memory is associated with sustained neural activity in cortical and subcortical structures. In particular, recent studies have suggested that the posterior parietal cortex plays a key role in maintaining mnemonic traces. However, it is not understood how the neural activity in these regions supports the maintenance of short-term memory. *The goal of this project is to develop a short-term memory task for head-fixed mice and to leverage recent advances in 2-photon calcium imaging and optogenetics to dissect the neural circuits underlying short-term memory.*

EISAI COMPANIES (JAPAN)
Mary Ann Jordan 1/24/14-1/23/16 $601,767
Mechanisms of microtubule-targeted drug-induced neuropathy

Neuropathy induced by the clinical treatment of cancer with microtubule-targeted drugs such as taxanes, ixabepilone, and vinca alkaloids is a major and highly debilitating dose-limiting toxicity. The novel halichondrin, eribulin, shows significant clinical efficacy along with a reduced incidence of peripheral neuropathy as compared with that produced by other microtubule-targeted drugs. The molecular and cellular mechanism(s) underlying the reduced incidence of neuropathy are poorly understood. Neuropathy typically develops gradually and is cumulative over the course of drug treatment. Among the neuronal effects that accompany peripheral neuropathy are changes to axonal structure involving microtubule organization, as well as impaired mitochondrial activity. These effects can lead to neuronal oversensitization, axonopathy, and eventually neuronal cell death.

For the past 2 years, we have been collaborating with Dr. Barbara Slusher's group at Johns Hopkins University to examine cellular and molecular effects of eribulin, vincristine, paclitaxel and ixabepilone that occur coincident with peripheral neuropathy in mouse models. These experiments involved examining the sciatic nerves of mice treated for two weeks with the maximum tolerated dose of each drug. Using immunostaining/light microscopic strategies, the most important results of this work are (i) the discoveries that nerves from paclitaxel and eribulin-treated mice exhibit significant decreases in axon density and a marked disruption of normal myelin morphology, (ii) the discoveries that eribulin-treated mice exhibited increased expression of axonal tubulin, acetylated tubulin, tyrosinated tubulin and the microtubule (+) end binding protein EB1, and finally, (iii) the discovery that microtubule-based transport of mitochondria in neuronal cells is inhibited more strongly by paclitaxel, ixabepilone and vincristine than by eribulin, at their relevant chemotherapeutic concentrations.

Here, we propose to expand upon our initial molecular and cellular analyses of MT-targeted drug effects on peripheral neuropathy, driving toward more mechanistic investigations and continuing our collaboration with Dr. Slusher's lab at Johns Hopkins. other microtubule-targeted drugs. The molecular and cellular mechanism(s) underlying the reduced incidence of neuropathy are poorly understood. Neuropathy typically develops gradually and is cumulative over the course of drug treatment. Among the neuronal effects that accompany peripheral neuropathy are changes to axonal structure involving microtubule organization, as well as impaired mitochondrial activity. These effects can lead to neuronal oversensitization, axonopathy, and eventually neuronal cell death.

CAL INSTITUTE FOR REGENERATIVE MEDICINE (CIRM)
Lina Kim 6/1/2012-12/1/2015 $61,580
Research Mentorship Program – Immersing High School Students in College Research

Entering its seventeenth year at the University of California, Santa Barbara (UCSB), the Research Mentorship Program (RMP) is a hands-on program for highly motivated high school students interested in participating in academic research in the arts, music, dance, social, life, physical sciences or engineering. During their six intensive weeks on campus, participants enroll in two credit bearing UCSB interdisciplinary courses – one a lecture course in which

students learn to analyze papers and write their own, evaluate research presentations and present their own research in a culminating symposium, and a second course in which students engage in aspects of on-going research with a UCSB faculty or one of their research team members as a mentor. Depending on the nature of the project, lab hours may range from 30 to 40 hours a week, occasionally working into the night when needed. Students select their mentors after approximately 50 potential projects are presented to the class by UCSB researchers. From these, each student choses 8 preferred projects. One of these researchers will be assigned and act as the mentor while the pre-college students work on aspect(s) of the mentor's research for the rest of the program. Participants will work in the lab, library and/or in the field, and be guided in research techniques, learning how to collect and analyze data, how to write a research paper and how to present findings in a public research symposium.

CAL H&W PUBLIC HEALTH, DEPARTMENT OF (CDPH)
Kenneth Kosik 7/1/13-6/30/16 $212,616
Detection of Genetic Factors which Modify the Age of Onset of Alzheimer's Disease

We will analyze the full genomes from members of a large Colombian family with familial Alzheimer's disease. Among the samples are patients that had the onset of dementia as early as age 39 and other patients with onset of dementia as late as age 67. From this genomic information we will search for genetic variants that are associated with outlier status in age at onset. These individuals have also contributed fibroblasts from which we can make neurons and use the neurons to identify the specific genes, which may be differentially expressed in neurons of the outliers. Finally we will determine whether any of the changes we observe in the DNA of these individuals corresponds to abnormal expression of the same gene in the neurons we are concurrently analyzing. This approach is a powerful means to discover genes that modify the age at onset of Alzheimer's disease and in some cases those genes may be suitable targets for treatment.

DOD ADVANCED REEARCH PROJECTS AGENCY (DARPA)
Kenneth Kosik 7/1/15-9/30/16 $819,423
A New Tool for Local Manipulation of Neuronal Micro-Circuitry with Ions and Force

We will build a tool that can reveal brain micro-circuitry, e.g. functional modules that act as elementary processing units bridging single cells to systems and behavior (Grillner and Graybiel, 2006). None of the currently available tools such as optogenetics, calcium dye imaging or multiple-tetrode recordings have the capability to reveal circuitry at multiple cellular scales, reversibly interrupt specific connections, locally deliver pharmacological probes, globally disrupt circuitry through direct access to a fluid interface all with a precise time stamp and while simultaneously visualizing specific cells under study. The neural circuit probe (NCP) that we propose is a deceptively simple device with all these capabilities. Although based on existing technologies such as multi-electrode arrays, atomic force microscopy and scanning ion conductance microscopy, the proposed instrument is an innovative disruptive departure from any existing instrument.

AMERICAN FEDERATION –AGING RESEARCH (AFAR)
Kenneth Kosik 6/1/2016-5/30/2017 $19,000

SIMONS FOUNDATION (SFARI)
Kenneth Kosik 11/1/2015-10/31/2016 $59,000

BAZ1B Haploinsufficiency and the Neuro-phenotypes of Williams Syndrome

The genomic segment deleted in Williams syndrome (WS) offers a unique opportunity to understand autism (1,2). Symmetrically divergent copy number variations of 7q11.23 display symmetrically opposite phenotypes with regard to the social facets of brain function. Hemizygous deletions result in excessive friendliness, unreserved behavior around strangers, an engaging personality, striking verbal ability, and an affinity for music. Duplications of the region resemble autism spectrum disorder with impaired social interactions and language deficits. Thus this genomic region represents the genotypic source of a cognitive axis that determines multiple facets of human social capabilities. To explain this phenotype, we determined the transcriptomes of induced pluripotent stem cell (iPSC)-derived neurons from WS patients and controls. Haploinsufficiency of the ATP dependent chromatin remodeler, BAZ1B (3) can explain nearly half of the brain

related transcriptomic dysregulation. Our interpretation of these changes is that the Williams micro-deletion results in a selective delay of neuronal maturation by creating a constriction at the point of cell cycle exit and, thereby affects the formation of brain micro-circuitry. We will test the former part of the premise.

SANTA BARBARA COTTAGE HOSPITAL
John Lew 5/21/13-5/20/16 $15,000
Development of p25 as a novel breast cancer therapeutic

CDK5/p25 is a neurotoxic protein that causes neuronal cell death. Our laboratory has shown that expression of p25 in all cells including tumor cells causes robust cell death, because while neurons specifically express p25, all cells express CDK5. The goal of this study is to develop a delivery system whereby p25 may be specifically targeted to breast cancer tumors, where
its toxic action is anticipated to kill tumor cells. Our collaborator, Dr. Erkki Rouslahti, has developed a tumor homing system in which a small peptide sequence, iRGD (CRGDKGPDC), is capable of both homing specifically to tumor vasculature and internalizing bacteriophage particles or nanoparticles coated with this sequence. In this proposal, we will test if p25 fused to iRGD can be targeted to breast cancer tumors in tumor-bearing mice. The goal is to test the specificity of targeting and the ability of p25-iRGD to penetrate tumors and cause tumor cell death. This study will be the first to test the specific tumor targeting of p25 by iRGD, and will provide the proof of concept that p25 homing to tumors may be a novel and viable strategy for a new cancer therapy.

UC IRVINE
Geoffrey Lewis 1/1/13-12/31/16 $1,023,806
Retinal progenitor cells for treatment of retinitis pigmentosa

Over the last year, in collaboration with a group at UCI, we have completed the pre-clinical work required by the FDA for a CIRM funded project using human retinal progenitor cells for the treatment of retinitis pigmentosa. Specifically we demonstrated that the progenitor cells slowed the loss of photoreceptors without causing any adverse effects in the eyes of the RCS rat, an animal model for this disease. These studies, completed at the NRI, helped lead to clinical trials of this treatment method in patients with retinitis pigmentosa beginning in August of 2015. The desired outcome is that the progenitor cells will protect surviving photoreceptors at the time of treatment from the further damaging effects of the inherited disease.

NIH GENERAL MEDICAL SCIENCES
Craig Montell 4/1/14-3/31/2016 $326,488
TRPA1: A Polymodal Sensor for Aversive Stimuli 7R01GM085335-06

The long-term goal of the research is to use the fruit fly, Drosophila melanogaster, as an animal model to unravel the mechanisms through which insects respond to sensory cues, ranging from changes in temperature to insect repellents. These questions are of potential relevance to the control of insect pests, since mosquitoes that spread diseases are attracted to humans through thermosensory, visual and chemical cues. Aversive temperatures and chemical repellents deter insects. Therefore, understanding the mechanisms underlying avoidance behavior may provide important insights into insect pest control. A key group of receptor proteins that sense environmental stimuli are Transient Receptor Potential (TRP) cation channels. Among the 13 Drosophila members, TRPA1 is of particular note as it is a detector for a wide array of noxious sensory inputs, including slightly warm or hot temperatures, insect repellents, and excessive light. Here, we propose to dissect the molecular, cellular and behavioral mechanisms through which TRPA1 allows larvae and adult flies to elude aversive stimuli. To accomplish our goals, we are employing a multidisciplinary approach, using a combination of molecular genetics, biochemistry, cell biology, electrophysiology and behavioral approaches.

NIH DEAFNESS & OTHER COMMUNICATION DISORDERS
Craig Montell 7/1/15-6/30/16 $322,926
Molecular Genetics of Contact Chemosensation 7R01DC007864-08

The long-term goal of this research project is to clarify the molecular mechanisms underlying the detection and discrimination of chemicals through contact chemosensation in the fruit fly, Drosophila melanogaster. Contact chemosensation allows flies to distinguish sweet from bitter molecules, as well as nonvolatile pheromones. Insect gustatory organs express a diversity of candidate molecular detectors. These include gustatory receptors (GRs), TRP channels, ionotropic receptors (IRs) and odorant binding proteins (OBPs), the latter of which promote the detection of chemicals by receptor proteins. However, the functions of most of these candidate gustatory receptors and binding proteins are unknown, or are understood poorly. This project is focusing on dissecting the mechanisms underlying contact chemosensation in flies using a multidisciplinary approach that includes electrophysiology, behavior, genetics, and cell biological approaches. During the last few years, the concept that GRs are required broadly for sensing sugars and bitter-tasting compounds has been confirmed. However, the biochemical functions of GRs are unclear. A long-term goal of this research is to apply the findings to the control of insect pests that spread disease.

NIH NATIONAL EYE INSTITUTE
Craig Montell 5/1/14-8/31/16 $376,075
Rhodopsins: from biosynthesis and degradation to unconventional functions

The goal of the research is to use the fruit fly, Drosophila melanogaster, as an animal model to unravel the molecular mechanisms underlying the biosynthesis, turnover and non-classical functions of rhodopsins. Rhodopsin is comprised of an opsin protein and a vitamin A-derived chromophore, which senses light. Among the most common forms of retinal degeneration are those that result from defects in the visual cycle (retinoid cycle)—an enzymatic pathway required for regeneration of the chromophore. Until recently it was thought that flies do not employ a visual cycle, since the chromophore does not normally release from photoactivated rhodopsin. However, some rhodopsin is internalized and the opsin gets degraded, thereby releasing the chromophore. We have recently made the discovery that flies use a visual cycle to regenerate the released chromophore. To accomplish our goals, we are employing a multidisciplinary approach using a combination of genetic, cell biological, electrophysiological, molecular and biochemical techniques. The long-term goals of these studies are to 1) uncover mechanisms underlying the retinal degenerations that result from defects in the visual cycle with the ultimate goal of discovering new therapeutic approaches, and 2) uncover the roles of the enigmatic extra-retinal opsins.

NIH- NATIONAL EYE INSTITUTE
Craig Montell 5/1/14-4/30/17 $383,750
Regulation of TRP channels and visual transduction

The long-term goal of this research project is to define the mechanisms through which the TRP channels in photoreceptor cells are activated and regulated in response to light. This project focuses on Drosophila phototransduction, which functions through a phospholipase C (PLC)-dependent signaling system, and culminates with Ca2+ and Na+ influx, via the TRP and TRPL channels. There exists a large family of mammalian TRPs, including channels in the intrinsically photosensitive retinal ganglion cells (ipRGCs) that are gated through a cascade that has notable parallels with fly phototransduction. The specific goals of this project are to answer major questions in Drosophila phototransduction concerning the mode of activation andregulation of the TRP channels. To accomplish our goals, we are employing a multidisciplinary approach, using a combination of molecular genetics, biochemistry, cell biology, and electrophysiology. The goal of aim 1 is to identify the molecule that directly gates the TRP and TRPL channels. Prior to activation of these channels, PLC causes hydrolysis of PIP2 to generate IP3, DAG and H+. However, despite the >20 years that have elapsed since the identification of the Drosophila TRP channels, the precise activation mechanism is not known. We recently identified a DAG metabolite that increased in concentration in a light-dependent manner.

We suggest that the studies that are the focus of this project are significant because they offer to resolve the mechanisms by which the TRP channels in photoreceptor cells are gated, localized and regulated. We also suggest that these studies will provide the framework for answering similar questions relevant to the channels in the ipRGCs, which contribute to light-induced circadian rhythms, sleep patterns and rudimentary image formation in the absence of rods and cones.

NIH- ALLERGY & INFECTIOUS DISEASES

Craig Montell 9/30/2015-7/31/2016 $767,500

Creation of a new generation of transgenic mosquitoes to control infectious disease

The most devastating infectious diseases worldwide are malaria and Dengue fever. Half the world's population lives in areas at risk for these diseases. According to WHO and the CDC, one billion people came down with malaria or Dengue last year. Despite widespread efforts to control malaria and Dengue, they remain enormous health problems affecting >100 countries, especially in Africa where ~500,000 children succumbed to these diseases each year. Clearly, current approaches to malaria and Dengue control have been inadequate. The latest approach to dealing with the spread of Dengue and other mosquito-borne diseases, is to release transgenic male mosquitoes bearing dominant mutations that, upon mating, render indigenous females either sterile or unable to reproduce the virus or parasite that causes dengue or malaria. However, the #1 obstacle to the success of these release strategies is that the transgenic males do not compete adequately with native males, greatly limiting the feasibility of this otherwise promising approach. We discovered mutations in several Drosophila signaling proteins that greatly increase male sex drive, and in at least one case this allows the males to outcompete wild-type males in mating. Since flies and mosquitoes are both Diptera, further investigation of these molecular pathways in flies may lead to successful strategies for improving the competitiveness of genetically modified mosquitoes, and ultimately make the release approach practical and save millions of lives. My laboratory is uniquely qualified to elucidate these molecular pathways using the fly as a model organism. It is essential to first fully define the signaling proteins and brain circuitry that normally serve to put the brakes on male sex drive in insects. This goal is uniquely achievable in Drosophila, since unlike any other insect, the fly enables us to apply with ease an unparalleled combination of electrophysiology, genetics, and cell biological approaches to identify genes and manipulate the activity of neurons. Once we decipher the mechanisms in flies, we propose to test the utility of our ideas in transgenic mosquitoes in open field trials. The potential future impact of these basic approaches goes beyond the control of insect borne diseases. One of the greatest challenges to addressing worldwide hunger is the destruction of crops by insect pests, especially in the Third World. Last year, crop destruction exceeded two trillion dollars worldwide. We propose that our creative line of investigation, offers the potential to lead to a revolutionary new strategy that will reduce the spread of insect pests that transmit widespread diseases and destroys agriculturally important crops, without the use of insecticides, insect repellents or other chemicals that might adversely affect human health.

NIH GENERAL MEDICAL SCIENCES

Denise Montell 7/1/2014-6/30/2016 $499,864

Developmental Regulation of Collective Cell Migration

NIH-NATIONAL EYE INSTITUTE

Benjamin Reese 1/1/2016-12/31/2016 $383,750

Demographics of Retinal Nerve Cell Populations

Populations of neurons vary in their demographics: They differ in their absolute numbers, in their intercellular spacing and the patterning this produces, in their degree of dendritic overlap and its regulation, and in their synaptic connectivity and the convergence ratios associated with their afferent neurons. The present research program has been addressing the causal relationships associated with such neuronal population dynamics, using the retina as a model system and working with a panel of twenty-six genetically distinct recombinant inbred (RI) mouse strains. Neuron number has been shown to vary considerably across these strains of mice, for twelve different classes of retinal neuron, and this variation maps to discrete genomic loci (quantitative trait loci, or QTL) for each cell type, showing minimal evidence for genomic co-regulation. The genetic sources of this variation in neuron number will be defined, for each cell type, and the developmental roles of these genes modulating cell number will be identified. Independent of neuron number, neurons vary in other histotypical features across these RI strains, including the orderliness by which they space themselves apart within a layer. The population of horizontal cells is one such example, where variation in the orderliness of their patterning maps to two narrow genomic loci. Causal genes and their variants at these loci will be pursued, and comparable spatial statistical analysis will be conducted for the other cell types to map QTL in pursuit of the genetic determinants of neuronal spacing. The consequence of such independent variation in the number of afferent and target neurons upon dendritic differentiation will also be examined, using the AII amacrine cell to explore the unique independent control of its lobular versus dendritic growth. Finally, a role for the transcription factor *Sox2* in cholinergic amacrine cells has recently been demonstrated, causing a mis-positioning of these amacrine cells between the inner nuclear layer and ganglion cell layer,

and a conversion of their mono-stratifying dendrites into a bi-stratifying morphology. The role of *Sox2* will be further explored to identify the downstream genes responsible for these altered cholinergic amacrine cell traits, by transcriptome-profiling of purified cholinergic amacrine cells from Sox2-deficient versus control retinas. The present research proposal will thereby identify the genetic determinants and intercellular interactions that underlie the demographic features of neuronal populations in the retina, clarifying our understanding of retinal development, as well as identifying genetic variants that may contribute to retinal disease.

NIH NATIONAL EYE INSTITUTE
Benjamin Reese 1/1/14-12/31/15 $348,046
Development of Retinal Bipolar Cells R01 EY019968

This research program is identifying the molecular and genetic determinants controlling the natural variation in nerve cell number, examining the populations of synaptically connected photoreceptors, bipolar cells and amacrine cells in the retina. We are also determining how such variation in afferent and target cell number modulates the dendritic morphology of the post-receptoral cells. This program will, consequently, clarify the developmental events and their underlying mechanisms that produce the functional architecture and connectivity of the retina. These studies will contribute to our understanding of retinal development and degeneration, and will enlighten our approach in developing treatments for retinal disease, particularly where the latter seek to re-establish connectivity following cell replacement therapy.

NIH CHILD HEALTH & HUMAN DEVELOPMENT
Joel Rothman 6/1/13-5/31/15 $269,676
Specification and Differentiation of Endoderm in C. elegans R01 HD062922

We are continuing our studies on how cell division and growth are controlled by investigating the cellular components that switch dividing cells into non-dividing cells with specialized functions. These processes are critically important in the genesis of cancer and are uncontrolled in growing tumor cells. The project is providing training for graduate students and undergraduate researchers who are learning molecular genetic and cell biological experimental methods that effectively address these problems.

NIH CHILD HEALTH & HUMAN DEVELOPMENT
Joel Rothman 4/1/2015-1/31/2020 $306,596
Developmental reprogramming and transorganogenesis

NIH CHILD HEALTH & HUMAN DEVELOPMENT
Joel Rothman 4/1/2015-1/31/2017 $297,865
Plasticity in an embryonic gene regulatory network

NIH CHILD HEALTH & HUMAN DEVELOPMENT
Joel Rothman
Mechanisms of Developmental Fidelity 7/1/2015-6/30/2016 $180,688

NIH
William Smith 4/1/2016-3/31/2017 $600,000
A Zeiss Lightsheet Microscope for Rapid Multi-Dimensional Imaging

NIH CHILD HEALTH & HUMAN DEVELOPMENT
William Smith 7/1/15-6/30/16 $317,827
Morphomic analysis of a simple chordate R01 HD059217

This proposed collaborative project will investigate fundamental processes driving chordate embryogenesis. The project will combine the skills and expertise of two research groups: one that works in the area of developmental biology, and the other in the area of image analysis and computer vision. The goal of the project is take a whole-embryo embryo approach to investigating morphogenesis in live embryos in all 4 dimensions (x,y, z and t). Specifically, we will collect and analyze confocal microscopy images to derive quantitative data on the division, shape, volume and movements of all cells in both selected developing organs and in whole embryos.

CALIFORNIA BLUEPRINT FOR RESEARCH TO ADVANCE INNOVATIONS IN NEUROSCIENCE (CAL-BRAIN)
William Smith 6/1/2015-5/31/2016 $120,000
Whole brain imaging in a primitive chordate

The formation of organs and tissues in the developing embryo requires coordinated action of multiple cells. Cells are not uniform structures, but rather have distinct sides, a property we call polarity. For example, cells in an organ may adhere tightly to a substrate with one face, while actively secreting on another face. We use a simple model organism in which the organs are composed of only tens to hundreds of cells to investigate the cellular mechanisms by which cells sense directionality and coordinate polarity while they assemble into organs.

ENDOCYTE INC.
Thomas Weimbs 5/1/2013-3/31/2017 $191,563
Pre-clinical efficacy of Folate-Conjugated mTOR Inhibitors and related compounds in Polycystic Kidney Disease

CIRM BRIDGES.
Thomas Weimbs 9/25/2015-9/16/2016 $3,300
CIRM Bridges Stem Cell Research Intern partnership with CSU Channel Islands

NIH DIABETES, DIGESTIVE & KIDNEY DISEASES
Thomas Weimbs 8/1/13-7/31/15 $184,607
A Novel Role of Syntaxin 3 as a Transcription Regulator R21 DK095248

NARE proteins mediate membrane fusion events in virtually all cellular membrane trafficking pathways. We have discovered an unexpected, novel function of the SNARE protein syntaxin 3 (Stx3). Stx3 normally has a C-terminal trans-membrane anchor and is involved in trafficking to the apical plasma membrane domain of polarized epithelial cells. We found that Stx3 undergoes cleavage at an extremely conserved glutamine residue which removes its trans-membrane domain resulting in a soluble fragment, Stx3(1-225). Furthermore, a novel splice-isoform of Stx3 (Stx3E) lacks the trans-membrane anchor, and is expressed in human kidneys. Both, the cleavage fragment and Stx3E (collectively called "soluble Stx3") bind to the nuclear import factor RanBP5, target to the nucleus and co-activate several transcription factors including ETV4. ETV4 is required for branching morphogenesis in kidney development, and associated with carcinogenesis and tumor metastasis. We found that kidneys from Autosomal Dominant Polycystic Kidney Disease (ADPKD) patients express a small Stx3 fragment – consistent with soluble Stx3. We hypothesize that cleavage and transcriptional regulation in the nucleus is a novel function that may be a common feature of syntaxin members of SNARE proteins. This may be a novel signaling mechanism that transduces information from cytoplasmic membrane trafficking events to the nucleus to affect changes in gene expression. If correct, this would introduce a new paradigm of SNARE function. More specifically, we hypothesize that soluble Stx3 plays a role in the regulation of renal epithelial morphogenesis, carcinogenesis and ADPKD.

NIH-DEAFNESS & OTHER COMMUNICATION DISORDERS
Yali Zhang 4/1/2016-3/31/2017 $153,500
Exploring the molecular and cellular basis of food texture sensation in Drosophila

Food texture, the physical properties of food such as hardness, softness and viscosity, plays an indispensible role in controlling an animal's taste preference. The texture of food is primarily detected through mechanosensory receptors located on the taste organ. Although food texture has enormous effects on food intake behavior, the molecular and cellular identities of mechanosensory receptors responsible for food texture sensation are largely unknown. Transmembrane channel-like (TMC) proteins comprise a cation channel family, which is highly conserved among species ranging from worms to flies, mice and humans. TMC appears to be widely used to control different forms of mechanosensation. For instance, both dominant and recessive mutations of TMC-1 result in severe hearing impairments in mice and humans. Moreover, our preliminary data suggested that fly TMC was likely to be a mechanosensor that is critical for discriminating foods on the basis of texture. In this proposal, the fruit fly will be used as a model organism to dissect the molecular and cellular mechanisms through which mechanical properties of food affect taste preferences. Firstly, a new physical approach will be developed to quantitatively measure how the taste organ responds to mechanical forces. Secondly, this proposal will employ Ca2+ imaging and temperature or light activation of specific neurons to study the function of *tmc*-expressing neurons. Lastly, this proposal will test if TMC is required and sufficient to be a mechanical sensor both *in vitro* and *in vivo*. Taken together, this proposal will use fly TMC as a novel tool to characterize the physiological functions of mechanosensory neurons that are critical for food mechanics sensation. Moreover, it will elucidate the force-gating mechanisms of TMC in fruit flies.

EISAI RESEARCH INSTITUTE
Leslie Wilson 7/2/2013-7/07/2016 $773,782
Functional Interactions between Eribulin and Microtubule +TIPS

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STATISTICAL SUMMARY FOR: (Neuroscience Research Institute)

		2015-16
1. Academic personnel engaged in research:		
a.	Faculty	29
b.	Professional Researchers (including Visiting)	8
c.	Project Scientists	15
d.	Specialists	34
e	Postdoctoral Scholars	31
f	Postgraduate Researchers	
	TOTAL	117
2. Graduate Students:		
a	Employed on contracts and grants	32
b.	Employed on other sources of funds	
c.	Participating through assistantships	
d.	Participating through traineeships	4
e	Other (specify)	
	TOTAL	36
3. Undergraduate Students:		
a.	Employed on contracts and grants	68
b.	Employed on other funds	
c.	Number of volunteers, & unpaid interns	11
	TOTAL	79
4. Participation from outside UCSB: (optional)		
a.	Academics (without Salary Academic Visitors)	6
b.	Other (local high school students/CIRM Creativity Program and Intern CSCI)	11
5. Staff (Univ. & Non-Univ. Funds):		
a.	Technical	22
b.	Administrative/Clerical	8
6. Seminars, symposia, workshops sponsored		15
7. Proposals submitted		59
8. Number of different awarding agencies dealt with*		46
9. Number of extramural awards administered		63
10. Dollar value of extramural awards administered during year**		29,128,659
11. Number of Principal Investigators***		46
12. Dollar value of other project awards ****		5,896,537
13. Number of other projects administered		62
14. Total base budget for the year (as of June 30, 2016)		342,089
15. Dollar value of intramural support		497,577
16. Total assigned square footage in ORU		14,584
17. Dollar value of awards for year (08 Total)		7,845,542

* Count each agency only once (include agencies to which proposals have been submitted).

** If the award was open during the year, even if for only one month, please include in total.

*** Number of PIs, Co-PIs and Proposed PIs (count each person only once.)

**** Other projects - such as donation, presidential awards, fellowships, anything that isn't core budget, extramural, or intramural.

ADVISORY COMMITTEE, ADMINISTRATIVE AND TECHNICAL STAFF

2015/16 NRI Advisory Committee

Mark Brezezinski, EEM Biology
Dennise Clegg, MCDB
Peter Coffey, NRI
Steve Fisher, NRI
Thomas Harriman, Community Member
Craig Montell, MCDB
Denise Montell, Chair, MCDB
Art Rosenblatt, Community Member
Janice Taylor, Development
Ty Vernon, Gevirtz Graduate School of Education

Ex-Officio Members
Stuart Feinstein, Co-Director NRI
Kenneth Kosik, Co-Director, NRI
Theresa Peña, Business Officer, NRI

NRI Administrative Staff

Judy Cushing, Personnel Manager
Azeb Demisse, Purchasing Manager
Max McCumber, Purchasing Manager
Kathleen McIntosh, Financial/Personnel Analyst
Theresa Peña, Business Officer
Bee Jay Yokoi, Contracts and Grants Manager
Angela Zeng, Assistant to Kenneth Kosik

Technical Staff

Elmer Guzman, Sequencing Facility Assistant Director
Cassidy Hinman, Stem Cell Lab, Associate Director
John Craighead, Computer Support
Geoffrey Lewis, Microscopy Support
Benjamin Lopez, Microscopy Director

Principal Investigators

Greg		Ashby	Professor	Psychology
Dennis		Clegg	Professor	NRII
Peter		Coffey	Researcher	NRI
Francis		Doyle	Professor	Chemical Engineering
Adele		Doyle	Asst. Researcher	NRI
Stuart		Feinstein	Professor	NRII
Steven		Fisher	Professor	NRII
Michael		Gazzaniga	Professor	Psychology
Michael	J.	Goard		
Scott		Grafton	Professor	Psychology
Michael		Gurven	Professor	Anthropology
Roger		Ingham	Professor	Speech & Hearing
Lincoln		Johnson	Researcher	NRII
Mary Ann		Jordan	Professor	NRII
Lina		Kim		Summer Sessions
Kenneth		Kosik	Professor	NRII
Tonya	Y	Kydland		NRI
Nichole		LaPointe	Postdoctoral Fellow	NRI
John		Lew	Professor	NRII
Geoff		Lewis	Researcher	NRI
Benjamin		Lopez	Postdoctoral Fellow	MECE
Zach		Ma	Professor	NRII
Michael		Mahan		
B		Manjunath	Professor	Electrical & Computer
Craig		Montell	Professor	MCDB
Denise		Montell	Professor	MCDB
Stanley		Parsons	Professor	NRII
Monte		Radeke		NRI
Benjamin		Reese	Professor	NRII
Joel		Rothman	Professor	NRII
Tal		Sharf	Postdoctoral Fellow	NRI
William		Smith	Professor	NRII
Tom		Soh	Professor	
James		Thomson	Professor	MCDB
Megan	T.	Valentine	Asst. Professor	MECE
Carol		Vandenberg	Professor	NRII
Thomas		Weimbs	Professor	NRII
Leslie		Wilson	Professor	NRII
Yali		Zhang		