Hollis T. Cline
The Scripps Research Institute
La Jolla, CA

Title

Exosome-mediated intercellular signaling in developmental and age-related disease models.

Abstract

Exosomes are thought to be released by all cells in the body and to be involved in intercellular communication, however the roles exosomes may play in neural circuits are largely unknown. We investigated exosome-mediated intercellular signaling in the context of neural circuit development and in age-related disease models. We examined neural circuit development in human iPSC derived neural cultures with MECP2 loss of function (LOF), a model of Rett Syndrome, which displays deficits in neural circuit development and function. Quantitative proteomics of neuronal exosomes indicate that aberrant circuit development resulting from MECP2 LOF leads to profound changes in exosome protein cargo related to neuronal proliferation, development and synaptogenesis. Treating MeCP2 LOF neural cultures with isogenic control exosomes rescues deficits in neuronal proliferation, differentiation, synaptogenesis and synchronized firing, whereas exosomes from MeCP2-LOF hiPSC neural cultures lack this bioactivity. These data indicate that exosomes convey signaling information required to regulate neural circuit development. Regarding age-related intercellular communication, in neurodegenerative diseases, including Alzheimer's disease (AD), inflammatory microglia to neuron signaling impairs neuronal function. *APOE4*, the strongest genetic risk factor for AD, alters both microglial activation and neuronal excitability. Using human iPSC-derived microglial and neuronal monocultures, we dissected how the *APOE* genotype in each cell-type independently contributes to inflammatory microglial regulation of neuronal activity. Microglia conditioned media or purified exosomes increase neuronal network activity. Inflammatory *APOE4* microglia increase neuronal hyperexcitability more than *APOE3* microglia, whereas neuronal *APOE* genotype plays a modest role in the response to microglia signaling. Interestingly, increased network activity increased lipid droplet metabolism and blocking lipid droplet metabolism abolishes network activity, linking microglia-driven hyperexcitability to neuronal metabolic stress. These findings illuminate how microglia-to-neuron communication drives inflammation-induced changes in neuronal circuit function, demonstrate a role for neuronal lipid droplets in network activity, and support a potential intercellular signaling mechanism through which *APOE4* increases neuronal excitability.

CURRICULUM VITAE

Hollis Tremaine Cline
Professor Emerita, Department of Neuroscience
Past Hahn Professor of Neuroscience
Past Director, Dorris Neuroscience Center
The Scripps Research Institute, CA
cline@scripps.edu

Dr. Hollis Cline has served as the Chair and Hahn Professor of Neuroscience in the Department of Neuroscience and the Director of the Dorris Neuroscience Center at The Scripps Research Institute until 2025. She received her Ph.D. in Neurobiology from the University of California, Berkeley. Holly moved to Scripps Research from Cold Spring Harbor Laboratory where she was Professor of Neuroscience for 14 years and served as Director of Research. She has received many accolades during her career including the National Institutes of Health Director's Pioneer Award, which she received in 2005 to launch a large-scale project to understand the architecture, development, and plasticity of brain circuits. In 2012, Dr. Cline was named a Fellow of the American Association for the Advancement of Science. This is an honor bestowed upon members by their peers. Dr. Cline's work was recognized "for seminal studies of how sensory experience affects the development of brain structures and function and for generous national and international advisory service to neuroscience." She has served as a Council member for the National Eye Institute and the National Institute of Neurological Disease and Stroke of the National Institutes of Health, and on the Blue Ribbon Panel for the National Institute of Child Health and Human Development. Dr. Cline was the President of the Society for Neuroscience in 2016. In 2022, Holly was elected to the National Academy of Sciences and the American Academy of Arts & Sciences. She is currently the AAAS Neuroscience Section Chair.

The goal of the research in the Cline lab has been to determine the mechanisms by which brain activity affects the development and function of the brain. The Cline lab has repeatedly been at the forefront of innovative technical advances, including in vivo time-lapse imaging, in vivo electroporation methods, viral gene transfer, serial section electron microscopy combined with in vivo time-lapse imaging (CLEM on steroids), whole animal electrophysiological recordings of visual responses and application of biorthogonal metabolic labeling of the activity-dependent nascent proteome. Studies in the Cline lab have made extensive use of Xenopus tadpoles, an experimental system amenable to multidisciplinary approaches applied to the intact animal, as well as rodents, and human induced pluripotent stem cells (hiPSCs) to investigate mechanisms of brain development, plasticity and function. Her studies have led to increased understanding of mechanisms controlling neurogenesis, synapse formation and plasticity, structural development of neurons and assembly of functional circuits. Her research has led to the discovery that neuronal activity regulates the development of the visual system through a variety of mechanisms, including changes in neuronal structure, synaptic strength, synaptogenesis and gene/protein expression. Dr. Cline's studies have relevance to healthy development and aging, as well as a variety of neurological disorders which result from errors in the development of brain circuitry.

EDUCATION

1977	B.A. Biology, Bryn Mawr College, Bryn Mawr, PA Honors Thesis: Characterization of the High Affinity Choline Uptake System in the Antennal Lobe of Manduca sexta. Advisor: D.J. Prescott
1985	Ph.D. Neurobiology, University of California, Berkeley, CA

Thesis: "GABA-ergic Neurons in the Leech" Advisor: G.S. Stent

POST GRADUATE EDUCATION

1985-89	Postdoctoral Fellow, (Advisor: M. Constantine-Paton), Department of Biology, Yale University, New Haven, CT. The role of NMDA receptors in development of the topographic retinotectal projection.
1989-90	Postdoctoral Fellow, (Advisor: Richard Tsien), Department of Molecular and Cellular Physiology, Beckman Center, Stanford University Medical Center, Stanford, CA. Calcium imaging in optic tectal cell cultures.
1980	Student, Neurobiology of the Leech Summer Course, Cold Spring Harbor Laboratory.
1995	Student, Cloning of Neural Genes Summer Course, Cold Spring Harbor Laboratory.

EMPLOYMENT

1976	Student Research Assistant, (C. DeDuve), Lab of Biochemical Cytology, The Rockefeller University
1977-79	Research Assistant, (A. S. Schneider/M. Sonenberg) Department of Endocrinology, Sloan Kettering Memorial Cancer Institute
1990-1993	Assistant Professor, Department of Physiology and Biophysics, The University of Iowa, Iowa City, Iowa
1994 -1996	Assistant Professor, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York
1997-1998	Associate Professor, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York
1998-2008	Professor, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York
2000-2008	Charles and Marie Robertson Professor of Neuroscience, Cold Spring Harbor Laboratory
2002-2006	Director of Research, Cold Spring Harbor Laboratory
2008-	Hahn Professor of Neuroscience, The Scripps Research Institute, CA
2008-	Professor, Depts. of Neuroscience & Molecular Medicine, The Scripps Research Institute, CA
2009-	Adjunct Professor, Department of Neurosciences, School of Medicine. Member, Neurosciences Graduate Program, UCSD
2013-	Adjunct Professor, Graduate Program, Salk Institute for Biological Studies, La Jolla, CA
2016-	Director, Dorris Neuroscience Center, The Scripps Research Institute, La Jolla, CA
2016-	Chair, Dept. of Neuroscience, The Scripps Research Institute, CA

SELECTED TEACHING EXPERIENCE

2008-2009	Director, Neurobiology Course, Marine Biological Lab, Woods Hole, MA
2009, 2011	Lecturer, Developmental Neurobiology Course, the Rockefeller University
2014-2018	Lecturer, Neurobiology 200, UCSD Neuroscience Graduate Program
2011-2024	Lecturer, Fundamentals in Neuroscience, The Scripps Research Graduate School

HONORS, SERVICE AND AWARDS, partial list

1977	High Honors in Undergraduate Research, Bryn Mawr College
1979-84	NIH Training Grant, UC Berkeley Neurobiology Group
1985-88	National Eye Institute Fellowship
1991-94	McKnight Scholars Award in Neuroscience
1993-96	Klingenstein Fellowship
1994-96	Patterson Trust Award in Medically-related Research
1995-97	National Down Syndrome Society Salary Award
1998-2008	Charles & Marie Robertson Chair in Neuroscience, CSHL
2000-2005	Executive Committee, Watson School of Biological Sciences
2005-2010	NIH Director's Pioneer Award
2006-2013	Associate, Neuroscience Research Program, Neurosciences Institute
2010-2013	Secretary, Society for Neuroscience
2012-2015	Council Member, National Eye Institute
2012-2013	Member, Blue Ribbon Panel for review of NICHD Division of Intramural Research
2012-	Fellow, AAAS American Academy for the Advancement of Science
2013	TSRI Outstanding Mentor Award
2014	IRP Review Committee, National Institute of Child Health and Human Development
2014-2020	Human Cell Types Advisory Council, Allen Institute for Brain Science
2014-2015	President-Elect, Society for Neuroscience
2015-2016	President, Society for Neuroscience
2018-2021	Council Member, National Institutes of Neurological Disease and Stroke
2019	Mika Salpeter Lifetime Achievement Award
2022	Member, National Academy of Sciences
2022	Member, American Academy of Arts and Sciences
2022-	Board Member, Coalition for Life Sciences
2023-	Scientific Advisory Board Member, Aligning Science Across Parkinsons
2024-2027	AAAS Neuroscience Section Chair

PROFESSIONAL AFFILIATIONS

Society for Neuroscience
American Academy for the Advancement of Science
American Society for Cell Biology
American Physiological Society
Dana Alliance for Brain Initiatives
Faculty for Undergraduate Neuroscience
Coalition for Life Sciences
Aligning Science Across Parkinsons

AREAS OF RESEARCH INTEREST

Analysis of the activity-dependent control of cell proliferation, neuronal development and circuit formation and function in the visual system using gene manipulations, in vivo structural and functional imaging, electrophysiology, genomics, proteomics and behavioral approaches.

PERSONNEL CURRENTLY SUPERVISED:

GRADUATE STUDENTS:

Ana Verduzco	Ph.D. Student	Skaggs Graduate School, TSRI
Yi Xie	Ph.D. Student	Skaggs Graduate School, TSRI

STAFF SCIENTISTS:
Masaki Hiramoto, Ph.D.
Caroline McKeown, Ph.D.

RESEARCH ASSOCIATE:
Na Na

TRAINEES PREVIOUSLY SUPERVISED

POSTDOCTORAL FELLOWS		YEARS	CURRENT POSITION	INSTITUTION
Carlos	Aizenman, Ph.D.	(2000-2004)	Professor	Brown University, Department of Neuroscience
Colin	Akerman, Ph.D.	(2000-2004)	Professor	University of Oxford, Department of Pharmacology
Jennifer	Bestman, Ph.D.	(2002-2012)	Assistant Professor	College of William and Mary
Isabel	Cantallops, Ph.D.	(1997-2002)	Patent Attorney	Clifford Chance Law Firm
Loraine	Campanati, Ph.D.	(2020-2021)	Senior Scientist	Fate Therapeutics
Jorge	da Silva, Ph.D.	(2005-2007)	Management Consultant	McKinsey & Company, Inc.
Marc	Davenne, Ph.D.	(1999-2002)	Postdoctoral Fellow	Centre National de la Recherche Scientifique
James	Demas, Ph.D.	(2004-2009)	Assistant Professor	St. Olaf College, Physic Dept. Northfield, MN
James	Edwards, Ph.D.	(1994-1997)	Pharmacist	Sirtex Medical
Regina	Faulkner, Ph.D.	(2009-2018)	Project and Alliance Manager	Takeda
Lisa	Foa, Ph.D.	(1998-2001)	Professor & Chair	University of Tasmania, Dept. of Anatomy & Physiology
Abigail	Gambrill, Ph.D.	(2012-2018)	Research Scientist	University of Washington
Kurt	Haas, Ph.D.	(1998-2004)	Professor	University of British Columbia, Dept. of Cellular and Physiological Sciences
Haiyan	He, Ph.D.	(2008-2016)	Assistant Professor	Georgetown University, Biology Dept
Masateru	Hiramoto, Ph.D.	(2006-2014)	Staff Scientist	Scripps Research Institute
Lin-Chien	Huang, Ph.D.	(2011-2017)	Research Scientist	Janssen Research&Development
Jianli	Li, Ph.D.	(2003-2010)	Associate	Montefiore Medical Center
Caroline	McKeown, Ph.D.	(2009-2011)	Sr. Staff Scientist	Scripps Research Institute
Elly	Nedivi, Ph.D.	(1996-1998)	Professor	MIT, Picower Center for Learning & Memory
Indrani	Rajan, Ph.D.	(1996-1998)	Director of Biology	Algos Inc

Edward	Ruthazer, Ph.D.	(1998-2005)	Professor	McGill University, Montreal Neurological Institute and Hospital
Lucio	Schiapparelli, Ph.D.	(2007-2015)	Research Scientist	Duke University
Pranav	Sharma, Ph.D.	(2004-2018)	CSO	Xosomix
Wan Hua	Shen, Ph.D.	(2006-2012)	Investigator	Hangzhou Normal University
Wun Chey	Sin, Ph.D	(1998-2001)	Research Associate	University of British Columbia, Dept. of Cellular & Physiological Sciences
Vatsala	Thirumalai, Ph.D.	(2003-2008)	Professor	National Centre for Biological Sciences, Bangalore, India
Christopher	Thompson, Ph.D.	(2011-2016)	Assistant Professor	Virginia Tech
Gang-Yi	Wu, Ph.D.	(1994-1997)	Senior Principal Scientist	Pfizer Global Research & Development
Dong-Jing	Zou, Ph.D.	(1992-1999)	Res. Associate Professor	Columbia University, Dept of Biological Sciences

GRADUATE STUDENTS		**YEARS**	**CURRENT POSITION**	**INSTITUTION**
Kasandra	Burgos, Ph.D.	(2004-2008)	Account Executive	NuGEN Technologie
Shu-Ling	Chiu, Ph.D.	(2003-2008)	Postdoctoral Fellow	Johns Hopkins Medical School/HHMI (Huganir Lab)
Gabriela	Edwards	(2013, visiting grad student)	Postdoctoral Fellow	VIB Center for Brain & Disease Research, KU Leuven
Rebecca	Ewald, Ph.D	(2001-2007)	Manager, Business Development	Roche
Ashkan	Javaherian, Ph.D.	(1998-2004)	Staff Research Scientist III	The Gladstone Institute
Kendall	Jensen, Ph.D.	(1999-2006)	Senior Scientist	Center for Alzheimer's and Related Dementias, National Institute of Aging, NIH
Melissa	Lau, Ph.D.	(2010-2016)	Social Media Manager	Benchling
Han-Hsuan	Liu, Ph.D.	(2011-2017)	Post-doctoral Researcher	HHMI/University of California, San Francisco
Jane	Lee-Osbourne, Ph.D., M.D.	(2004-2009)	General Surgical Resident	University of Nebraska Medical Center
Zheng	Li, Ph.D.	(1998-2001)	Chief, Section on Synapse Plasticity	National Institute of Mental Health
Emiliano	Rial Verde, Ph.D.	(2000-2005)	Vice President	Bunge Loders Croklaan Nutrition @ Bunge

Sahil	Shah, Ph.D.	(2015-2019)	Resident	University of California, San Francisco Medical School
Ana	Verduzco	(2020-2025)		Skaggs Graduate School, TSRI
Sonia	Witte, Ph.D.	(1991-1995)	Freelance Scientific Consultant	Salt Lake City, Utah
Yi	Xie	(2019-2024)	Research Scientist	Neuroscrine

MASTERS STUDENTS		**YEARS**	**CURRENT POSITION**	**INSTITUTION**
Erika	Barth	(2018-2020)	Medical Student	University of California, Los Angeles
Vi	Dang	(2018-2019)	PhD Student	UC, Irvine
Aaron	Ta	(2018-2020)	Data Analyst	Illumina
William	Gaor	(2020-2021)		San Diego State University

POSTDOCTORAL & GRADUATE STUDENT SUPPORT

1997-1999	(Kurt Haas) Helen Hoffritz Trust “CaMKII and Synapse Development”
1999–2005	(Rial Verde, Emiliano) Howard Hughes Medical Institute, Predoctoral Fellowship, David and Fanny Luke Fellowship
1999–2002	(Davenne, Marc) Human Frontier Science Program Grant, "Functional Analysis of an Activity Regulated Gene in Visual System Neuronal Plasticity."
1999–2002	(Ruthazer, Edward) NIH Individual National Research Service Award (900100), "Mechanisms of Scotoma-Induced Retinotectal Plasticity."
2000-2001	(Lisa Foa) Helen Hoffritz Trust “Role of Homer in the Developing Visual System”
2000–2004	(Ewald, Rebecca) Watson School of Biological Sciences Engelhorn Scholarship
2001–2002	(Aizenman, Carlos D.) Epilepsy Foundation research training fellowship.
2001–2004	(Akerman, Colin J.) Wellcome Trust International Travelling Fellowship, "Role of GAB Aergic Neurons in the Formation and Maturation of the Retinotectal Synapse."
2002–2003	(Ruthazer, Edward) Helen Hoffritz Trust Grant, "Activity-Dependent Refinement of Topographic Maps in the Developing Visual System."
2002–2003	(Haas, Kurt) Epilepsy Foundation research training fellowship.
2003-2005	(Haas, Kurt) NARSAD Young Investigator Award “Control of Dendritic Development”
2003–2005	(Bestman, Jennifer) FRAXA Research Foundation, "FMRP-mediated Dendritic Protein Synthesis required for Correct Morphological Development in Neurons."
2003–2004	(Sharma, Pranav) Cold Spring Harbor Laboratory Association Fellowship
2004–2005	(Ruthazer, Edward) NARSAD Young Investigator Award, "In Vivo Time Lapse Imaging of Axon Structural Stabilization by Adhesive Interactions at the Synapse."
2005-2006	(Da Silva, Jorge Santos) EMBO Long-term Fellowship, “Activity-dependent mechanisms controlling GABAergic neuron development during functional circuitry formation”.
2006-2008	(Da Silva, Jorge Santos) HFSPO Long-term Fellowship, “Activity-dependent mechanisms of GABAergic neuron development during functional circuitry formation”.
2007-2010	(James Demas) NIH Individual National Research Service Award “ Presynaptic regulation of dendritic structural dynamics”
2009-2012	(Sharma, Pranav) California Institute for Regenerative Medicine (CIRM) “Activity-dependent regulation of neurogenesis”
2010-2013	(Lau, Melissa) NSF Predoctoral Fellowship

2011-2014 (Faulkner, Regina) NIH National Research Service Award "Experience-dependent development of neural circuits in the Xenopus visual system"
2011-2013 (Chris Thompson) California Institute for Regenerative Medicine (CIRM) "Hormone-dependent regulation of neurogenesis"
2012-2014 (Heidi Sharipov) CARTA Fellowship in Anthropogeny
2013-2016 (Abigail Gambrill) NIH National Research Service Award "Intertectal input shapes visual circuit development and post injury remodeling"
2013-2015 (Chris Thompson) NIH Pathway to Independence K99 Award "Effects of environmental thyroid hormone disrupting compounds on CNS development"
2013-2016 (Lin-Chien Huang) California Institute for Regenerative Medicine (CIRM)
2018-2021 (Ana Verduzco) Helen L. Dorris Fellowship
2021-2023 (Ana Verduzco) Diversity Supplement NIH EY031597

ADMINISTRATIVE DUTIES
Journal Reviewer: Cell Reports, Current Biology, Genes and Development, Journal of Comparative Neurology, Journal of Neurobiology, Journal of Neurophysiology, Journal of Neuroscience, Nature, Nature Neuroscience, Neuron, Neuroscience, Science.

Editorial Boards: Visual Neuroscience, Developmental Neurobiology, Neural Development, Journal of Comparative Neurology, Annual Review of Neuroscience

Recent Service on Committees:
The Scripps Research Institute
Faculty Search Committees
TSRI Academic Planning Committee, 2015-2016
TSRI Faculty Lecture Series Planning Committee, 2014-2019
Executive Advisory Committee, 2021-present

National Service, Selected
MIT External Advisory Committee, Picower Center for Learning and Memory (2000-07)
Program Committee, Society for Neuroscience (2001-2004)
Council, Society for Neuroscience (2002-2006)
AAAS Electorate Nomination Committee (2004-2006)
National Institute of Neurological Disorders & Stroke, Board of Scientific Counselors (04-07)
National Institute of Neurological Disorders & Stroke, Board of Scientific Counselors (Co-Chair 2005-2006)
Review Panel, NIH Director's Pioneer Award (2006-)
Keystone Symposia Neurobiology Study Group (2006)
NIH Blueprint Meeting on Neural Development (2006)
NIH Roadmap Meeting (2006)
AAAS Fellows Nomination and Election Committee (2007-2012)
Council, National Eye Institute (2012-2016)
Council, National Institute of Neurological Disorders and Stroke (2018-2021)

International Service:
Reviewer:
Wellcome Trust (U.K.)
Alberta Heritage Foundation for Medical Research (Canada)
Israel Science Foundation
Review Panel, Howard Hughes Medical Institute, Canadian & Latin America Competition, 2006

Contributions to Science

1. Mechanisms Controlling the Organization of Sensory Projections in the Brain. Sensory projections in the brain are highly organized into spatial representations of the sensory world, such as topographic maps or ocular dominance columns. In the mid-1980's, evidence indicated that patterned sensory input activity is required for the development of organized projections, but the mechanisms underlying activity-dependent control of topographic sensory map formation were not known. As a postdoctoral fellow with Martha Constantine-Paton, I demonstrated that NMDA receptors are required for development and maintenance of retinotopic maps and ocular dominance columns in the Xenopus tadpole retinotectal projection (Cline et al 1987). In my lab at Cold Spring Harbor Laboratory, we demonstrated that postsynaptic NMDA receptors on tectal neurons regulate axon arbor dynamics (Ruthazer et al, 2003). Together, this work demonstrated the role of NMDA receptors in activity-dependent map formation. Studies in other experimental systems and other sensory projections corroborated our findings (Constantine-Paton et al, 1990). This body of literature is largely interpreted as demonstrating that Hebbian coactivity rules govern map formation and was famously summarized as 'Neurons that fire together, wire together'. Objects moving through visual space stimulate neighboring retinal ganglion cells in a temporal sequence. We found that the temporal sequence of activity in the retina instructs the spatial organization of retinal axons in the brain, ie 'Neurons that fire in sequence, wire in sequence' (Hiramoto & Cline, 2014). This principle of a spatial to temporal to spatial transformation of information in the visual system may apply to other brain regions that encode space and time, such as the hippocampus.

2. Activity-dependent Mechanisms Regulating Synapse Maturation, Dendritic & Axonal Arbor Development. We have devoted considerable effort to understand the complex interplay by which activity-dependent mechanisms regulate neuronal development and plasticity in the context of an intact functional circuit. In the 1990's we established methods for time-lapse in vivo imaging of developing neurons in Xenopus tadpoles using laser scanning confocal microscopy combined with single cell fluorescent labeling. We used these state-of-the-art methods to analyze the structural dynamics that occur during dendritic and axon arbor development in intact animals. We discovered that dendritic arbor dynamics are regulated by visual stimulation and identified activity-dependent structural plasticity mechanisms (Sin et al 2002). We were the first lab to use 2 photon time-lapse imaging to identify cellular and molecular mechanisms underlying structural plasticity (Ruthazer et al 2003). We developed viral gene transfer and electroporation methods to regulate gene expression in tadpole neurons in vivo, allowing us to manipulate activity-dependent signaling molecules, such as CaMKII, and show that glutamatergic synaptic activity and downstream CaMKII activity regulate the elaboration of neuronal dendrites by controlling the dynamic rates of branch additions and retractions (Wu & Cline, 1996). By incorporating electrophysiological assays of synapse formation and maturation with data on structural plasticity, we demonstrated the integral interaction between functional synaptic plasticity and structural plasticity (Wu et al 1998). To establish a direct relation between structural plasticity seen at the light microscope level and ultrastructural rearrangements in synaptic connectivity, we conducted a tour-de-force series of experiments in which we collected time-lapse in vivo 2 photon imaging data demonstrating structural plasticity of dendrites over hours and days, followed by complete serial section transmission electron microscope reconstructions of the same neurons (Li et al, 2011). These datasets demonstrated a startling degree of synaptic rearrangements that occur in dynamic dendritic branches and highlighted the magnitude of synaptic loss and synaptic consolidation that occur during microcircuit refinement. They also set an elevated standard for correlated analysis of in vivo synaptic dynamics and serial section EM analysis of the complete dendritic arbor. The plasticity mechanisms we identified in Xenopus are conserved across vertebrate species including mammals. These foundational studies are now the heart of the modern framework for activity-dependent control of neuronal development and circuit plasticity.

3. The Function of Activity Regulated Genes in Brain Circuit Development. Hundreds of genes are induced by activity in the brain, however the functions of activity-regulated genes in the intact brain were poorly understood. We collaborated with Paul Worley and Elly Nedivi, both of whom had conducted early screens to identify activity-induced neural genes, to analyze the cellular and molecular functions of activity-regulated genes/proteins in neuronal development, plasticity and circuit assembly in a highly assessable vertebrate system. These studies, including those mentioned below, demonstrated that activity-regulated mechanisms impinge on circuit development by regulating such diverse cellular events as AMPAR trafficking, axon pathfinding, dendritic arbor development, cytoskeletal dynamics and protein synthesis. We have demonstrated the function of several activity-induced genes and activity-regulated proteins in neuronal and circuit development, starting with our early studies on the role of αCaMKII in dendritic arbor development, glutamate receptor trafficking and synaptic maturation, mentioned above. This work has contributed to basic understanding of the iterative role of activity and activity-induced gene and protein transcription/translation in circuit development.

4. The Activity-dependent Nascent Proteome. The brain processes information, makes decisions, mediates cognitive and motor outputs through the functions of proteins in different types of connected neurons organized in complex circuits. Although experience-dependent transcriptional dynamics have been extensively studied, comparable knowledge about proteome dynamics is woefully incomplete. Current estimates indicate that there are 21,000 genes and 250,000 - 1 million different proteins in humans. An important next step in understanding brain plasticity and function is to generate an accurate knowledgebase of proteins in the intact brain, their subcellular distribution and how they change in response to activity and sensory input. In collaboration with Dr. John Yates, at the Scripps Research Institute, a world-renown expert in mass spectrometric methods, we developed sensitive methods to query changes in proteins that are applicable to intact animals (Schiapparelli et al, 2014). To identify activity-induced changes in brain proteomics that might contribute to brain function and plasticity, we conducted unbiased quantitative proteomic screens of newly-synthesized proteins in response to visual stimulation in Xenopus and rodents. We used metabolic protein labeling which uses incorporation of a noncanonical amino acid methionine analog followed by click chemistry tagging with biotin, to label newly synthesized proteins (Shen et al 2014, Liu et al 2019). Our studies in Xenopus provided the first in vivo analysis of visual experience dependent nascent proteomic dynamics and importantly, analyzed novel candidate plasticity proteins (CPPs) whose function in experience-dependent neuronal, circuit and behavioral plasticity had not been previously recognized. Expanding our studies to rodents enabled us to identify an extensive array of neuronal cell-type specific changes in activity-regulated nascent proteins (Schiapparelli et al 2022, Xie et al 2025). Our proteomic analysis of newly-synthesized proteins identified strong regulation of proteostasis-related proteins, including Emerin, which surprisingly acts as activity-regulated rheostat controlling proteostasis across a range of neuronal activity levels by gating protein synthesis. We anticipate that further examination of the nascent proteomic datasets will reveal novel insights into brain function, development and aging. Indeed, our current studies take advantage of our state-of-the-art proteomic pipeline to examine age- and Alzheimer's disease-related changes in proteostasis mechanisms by analyzing dynamics of brain activity induced newly-synthesized proteins.

5. Exosome-mediated intercellular signaling in brain circuit development and aging. Brain circuit function takes advantage of diverse mechanisms for intercellular signaling. Since Simon Levay first used radioactive amino acid injection into eyes to reveal ocular dominance columns in visual cortex of non-human primates and other mammals, the potential transfer of proteins between synaptically connected neurons in the CNS has been a provocative mystery. Using retinal protein labeling strategies and mass spectrometry, we discovered hundreds of proteins are transported between neurons in the healthy adult visual system and that the likely mechanism of transport is by exosomes

(Schiapparelli et al 2022). We wrote a perspective about exosomes, in which we postulated that extracellular vesicles, including exosomes, signal during brain development (Sharma et al 2013) and pursued this question in an hIPSC model of Rett Syndrome, an inherited type of autism spectrum disease. To examine exosome-mediated signaling events, we collaborated with Dr. Alysson Muotri, at UCSD, an expert in use of hIPSC to investigate human neurodevelopmental diseases. We conducted a quantitative comparative proteomic analysis of exosome cargo released from hiPSC-derived neural cultures from Rett patient cell line or CRISPR corrected controls. The analysis demonstrated a significant enrichment in neurogenic proteins in control exosomes compared to a paucity of neurogenic proteins in exosomes from Rett neural cells. We validated the proteomic data with extensive imaging-based assays of exosome bioactivity in human cells. Control neural exosomes exhibit neurogenic bioactivities, including increased cell proliferation in vivo and in vitro, increased synaptogenesis and increased neural network activity (Sharma et al 2019). This work indicates exosomes play diverse roles pertaining to intercellular signaling, inspiring us to pursue in their function in the context of neurodegenerative diseases, as mentioned below.

6. Microglia-to-Neuron Signaling Links APOE4 and Inflammation to Enhanced Neuronal Lipid Metabolism and Network Activity. Microglial signaling to neurons beneficially regulates neuronal circuit development and plasticity, but microglia-mediated neuroinflammation contributes to the onset and progression of neurodegenerative diseases, including Alzheimer's disease (AD). Interestingly, expression of *APOE4*, the strongest genetic risk factor for AD, affects both microglial activation and neuronal excitability, highlighting the interplay between lipid metabolism, inflammation, and neuronal function. It remains unclear how microglial inflammatory state is conveyed to neurons to affect circuit function and whether *APOE4* expression alters this intercellular communication. We addressed these questions using a reductionist model of human iPSC-derived microglial and neuronal monocultures to dissect how the *APOE* genotype in each cell-type independently contributes to microglial regulation of neuronal activity during inflammation. Conditioned media (CM) from LPS-stimulated microglia increased neuronal network activity, assessed by calcium imaging, with *APOE4* microglial CM driving greater neuronal activity than *APOE3* CM. CM-derived exosomes from LPS-stimulated microglia mediate increases to network activity, identifying a. Interestingly, increased network activity is accompanied by increased lipid droplet (LD) metabolism and blocking LD metabolism abolishes network activity. These findings illuminate how microglia-to-neuron communication via exosomes drives inflammation-induced changes in neuronal circuit function, demonstrate a role for neuronal LDs in network activity, and provide evidence for a mechanism through which *APOE4* increases neuronal excitability.

7. Brain-based AI. Artificial intelligence (AI) mediated recognition of natural movie scenes lags behind static image recognition. Brain-based principles of visual system function have contributed to computer science-based static image recognition technologies, suggesting that brain-based strategies for encoding motion may aid AI movie recognition. We discovered movie encoding principles in the Xenopus visual system, including how image sequences and time duration are encoded and how visual experience-induced plasticity modifies movie detection features. We developed movie recognition algorithms based on the brain-based movie encoders that exceed human capability for distinguishing movie stimuli, and importantly, reduce data size and processing (Hiromoto & Cline, 2024). This study demonstrates that brain-based movie processing principles enable efficient machine learning, addressing major weaknesses in current AI approaches.