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BIOGRAPHICAL SKETCH

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NAME: Jason Aoto

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POSITION TITLE: Assistant Professor

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable. Add/delete rows as necessary.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	Completion Date MM/YYYY	FIELD OF STUDY
University of California, Los Angeles	BS	07/2002	Biochemistry
University of California, Berkeley	Ph.D.	12/2009	Molecular and Cellular Biology (Neuroscience)
Stanford University (Postdoc)	na	12/2015	Molecular and Cell Physiology

A. Personal Statement

I believe that I am in an unique position to perform the first dissection of the ventral subiculum microcircuit with molecular, cell-type specific and synapse specific resolution. To accomplish this goal, I will employ a multidisciplinary approach, which consists of mouse genetics, molecular biology, electrophysiology, stereotaxic injection of virus and single-cell RNA-sequencing. I received significant training, first at UC Berkeley as a graduate student and later at Stanford University as a postdoctoral fellow, in the application and execution of modern approaches to address fundamentally important questions in neuroscience.

As a graduate student in Dr. Lu Chen's lab at UC Berkeley, my thesis work focused on the molecular mechanisms underlying synapse formation and homeostatic synaptic plasticity. Dr. Chen is recognized in the field of AMPA-receptor trafficking and homeostatic plasticity. In the Chen lab, I became proficient in primary culture electrophysiology to assess how molecular manipulations alter synaptic transmission properties. I utilized these techniques in two major discoveries from the lab. First, I identified the mechanism and characterized the molecule that is selectively involved in glutamatergic synapse formation on dendritic shafts. Excitatory synapses formed on dendritic shafts play a significant role during development and are predominately found on most GABAergic interneurons in the brain. By providing the first molecular handle on excitatory shaft synapses, we can now investigate the physiological function of this elusive type of synapses. Second, and probably more importantly, I identified all-trans retinoic acid and its receptor, retinoic acid receptor alpha, as the key regulators of activity-dependent translation of a select subpopulation of dendritically targeted mRNAs. Surprisingly, we found that the classical nuclear transcription factor, RARa, is targeted to dendrites and acts as a translational repressor of mRNAs that encode for key synaptic proteins. Following activity blockade, RA secretion is increased, binds to RARa and de-represses translation of GluA1 to globally strengthen synaptic strength. To the best of our knowledge, the mechanism that we discovered is the first example of a ligand-gated translation factor. My work on RA and RARa performed in Dr. Chen's laboratory is currently the focus of two NIH-funded grants.

In 2009, I joined the laboratory of Dr. Thomas Südhof at Stanford University as a postdoctoral fellow. Dr. Südhof is a internationally-recognized expert in the field of presynaptic vesicular release and in the molecular biology of neurexins. In his laboratory, I received training in mouse genetics, the biology of synaptic cell-

adhesion molecules and synaptic electrophysiology. With my collaborator, Dr. Robert Malenka, a leader in slice electrophysiology, synaptic plasticity, striatal circuitry, viral circuit tracing and optogenetics, I became highly experienced in *ex-vivo* hippocampal acute slice electrophysiology and synaptic plasticity. Being surround by a confluence of expertise at Stanford University, I utilized the multidisciplinary training that I received from Drs. Thomas Südhof and Robert Malenka to interrogate the synaptic function of neurexin-3 (Nrxn3). Discovered over 20 years ago as the endogenous receptor for the venom of the black widow spider. There are three neurexin gene, with each gene giving rise to two major gene products (alpha and beta). Each gene product is subject to extensive alternative splicing at up to six conserved splice sites. Biochemically, neurexins have been shown to be essential presynaptic cell adhesion molecules, however, their function at the synapse was uncharacterized. The two main questions that I set out to address as a postdoctoral fellow were: 1.) does a single neurexin gene's products have a significant role at the synapse and 2.) does alternative splicing encode for synaptic properties of these molecules. I addressed these questions by taking a genetic, molecular and electrophysiological approach *in vitro* and in *ex vivo* slices. First, I found that neurexin-3 has distinct dominant, non-redundant functions that are cell-type and brain region specific (hippocampus vs. olfactory bulb). Moreover, I found that brain-region specific genetic manipulations of neurexin-3 expression significantly altered animal behavior. Second, I investigated the function of alternative splicing at the conserved splice site #4 (SS4) in neurexin-3 using a novel mouse model. I found that the genetic manipulation of the SS4 alternative insert in presynaptic neurexin-3 altered postsynaptic AMPA-receptor strength in the subiculum, but seemingly does not play a role in the olfactory bulb. Taken together, my work on neurexin-3 provided the first insight into some of the possible synaptic functions of a single neurexin gene and of alternative splicing at splice-site #4. Moreover, my published work provided a conceptual advancement in the field by providing evidence that individual neurexins can have distinct, non-overlapping functions at the same synapse, while also collectively functioning in other core synaptic roles. To this point, in collaboration with a colleague, I contributed to the findings that the neurexin-1β, -2β and -3β in the hippocampus are redundantly required to maintain proper endocannabinoid tone at excitatory synapses in the hippocampus.

Together, I believe that I am well-trained to investigate dissect the ventral subiculum microcircuit on a cell-type specific, molecular and synaptic level to explore 1.) how the disease-relevant subicular microcircuit is functionally connected and organized, 2.) how neurexin-3, a gene frequently altered in humans suffering from Schizophrenia and drug addiction, functions in a synapse-specific manner and 3.) how acute drug administration alters synaptic properties of the microcircuit in the context of neurexin-3.

B. Positions and Honors

Positions and Employment

2002-2004 Staff Research Assistant, University of California, Irvine
2009-2015 Postdoctoral Fellow, Südhof Laboratory, Molecular and Cellular Physiology, Stanford University
2016- Assistant Professor, Dept. of Pharmacology, University of Colorado Denver

Other Experience and Professional Memberships

Alpha Chi Sigma: Professional Chemistry Fraternity (1999-)

Society for Neuroscience (2005-)

Molecular Psychiatry Association (2013-)

Invited Seminar Speaking Engagements

Stanford Institute for Neuro-Innovation & Translational Neurosciences Annual Retreat (2011)

First Annual Molecular Psychiatry Association Meeting (2013)

C. Contribution to Science

My pre-doctoral work in the laboratory of Dr. Lu Chen focused on the molecular mechanisms underlying synapse formation and homeostatic plasticity. In her laboratory, my work provided two significant contributions: 1. I found that in certain excitatory synapses in the hippocampus *in vitro* and *in vivo*, ephrinB3, commonly thought to be a presynaptic adhesion molecule, is localized to the postsynaptic density and functions to selectively promote excitatory synapse formation on dendritic shafts. The neuroscience field generally studies excitatory synapses made onto dendritic spines - small, actin-rich protrusions that extend from the dendrite -

due to the unique (and obvious) structure, abundance in the adult hippocampus (>90% of excitatory synapses form on spines in the adult) and the molecular tools to selectively manipulate spine morphology. Excitatory shaft synapses are less studied, due in part to the fact that they are visually difficult to distinguish without immunostaining and the severe lack of molecular tools to manipulate formation. However, this does not mean that shaft synapses are unimportant because they are predominantly found in the developing hippocampus and are extensively found in the cortex of adult brains. Moreover, almost all GABAergic interneurons receive excitatory input directly onto their dendritic shafts. My findings provide the first molecular handle on shaft synapse formation and allow the field to investigate the physiological function of these synapses.

Aoto J, Ting P, Maghsoodi B, Xu N, Henkemeyer M, and Chen L. (2007) Postsynaptic ephrinB3 promotes shaft glutamatergic synapse formation. *J. Neurosci*, 27: 7508-19. PMID: 17626212

Aoto J, Chen L. (2007) Bidirectional ephrin/Eph signaling in synaptic functions. *Brain Res*, 1184:72-80. PMID: 1766489

2. It is thought that in addition to Hebbian forms of plasticity (LTP/LTD), non-Hebbian forms of plasticity exist to stabilize neural networks to prevent runaway excitability or network silencing. One form of non-Hebbian plasticity is called homeostatic synaptic scaling. In response to chronic changes in network activity, neurons will globally increase or decrease their activity to promote stability. We were interested in studying the molecular mechanisms underlying this unique form of plasticity. In a series of work, we found that one form of homeostatic synaptic scaling involves all-trans retinoic acid signaling to activate the select translation of dendritically localized mRNAs. We surprisingly found that a fraction of retinoic acid receptor alpha (RARa), a canonical nuclear transcription factor, is localized to dendritic RNA granules to act as a translational repressor. Thus, when activity is blocked, retinoic acid secretion is enhanced, binds to dendritic RARa and de-represses translation of GluA1 mRNA to globally strengthen synapses. The impact of these papers is three-fold: 1.) they provided the first molecular mechanism to explain homeostatic synaptic plasticity; 2.) identified, to the best of our knowledge, the first ligand-gated translation factor; and 3.) demonstrated the possibility of specificity of translation of dendritically targeted mRNAs (despite the fact that RARa binds multiple target mRNAs, only GluA1 translation is up-regulated in RA-induced homeostatic synaptic scaling), which has implications in other forms of plasticity that require translational specificity of dendritic mRNAs. The work from this project is the focus of two major NIH-funded grants in the Chen lab.

Sarti F, Schroeder J, ***Aoto J***, Chen L. Conditional RARα knockout mice reveal acute requirement for retinoic acid and RARα in homeostatic plasticity. (2012) Front Mol Neurosci. PMID: 22419906

Aoto J*, Nam, CI*, Poon MM*, Ting P, and Chen L. (2008) Synaptic scaling by all-trans retinoic acid in homeostatic synaptic plasticity. *Neuron*, 60: 308-220. PMID: 18957222

*Equal Contribution

Maghsoodi B, Poon MM, Nam CI, ***Aoto J***, Ting P, and Chen L. (2008) Retinoic acid regulates RARa-mediated control of translation in dendritic RNA granules during homeostatic synaptic plasticity. *Proc Natl Acad Sci U S A*, 105: 16015-20. PMID: 18840692

3. As a postdoctoral fellow in Dr. Thomas Südhof's laboratory, I focused on interrogating the synaptic function of a family of trans-synaptic cell-adhesion molecules called the neurexins. The neurexins were discovered over 20 years ago as the presynaptic endogenous mammalian receptor of Black widow spider venom. Neurexins are expressed from three evolutionarily conserved, highly homologous neurexin genes (Nrxn1-3) with each gene producing an alpha and beta mRNA, which can undergo regulated alternative splicing at up to 6 conserved splice sites. In addition mutations in all three neurexin genes have been associated with neuropsychiatric disorders (e.g. ASDs, ADHD and schizophrenia). Interestingly, mutations to the human Nrxn3 gene have been most commonly associated with addiction disorders. Despite their essential function and their obvious importance in human disease, very little was known regarding the biological significance of an individual neurexin at the synapse and the functional consequences of regulating alternative splicing. Due to the unique association of Nrxn3 with drug addition AND neuropsychiatric disorders focused my efforts on neurexin-3 and used two different novel mouse models to study the function of the Nrxn3 gene and how alternative splicing at splice-site 4 regulate synaptic transmission. I found that the Nrxn3 gene is required for distinct synaptic properties in a brain-region specific manner. In the olfactory bulb, Nrxn3 is required to maintain GABAergic synaptic transmission while in the hippocampus; it is critical to stabilize postsynaptic AMPA receptors. Using a novel genetic strategy to selectively regulate the inclusion or exclusion of the splice-

site 4 alternative exon, I found that the presence or absence of the conserved 90 amino acid sequence alone can control postsynaptic AMPAR stability. By contrast to my findings in the olfactory bulb following the genetic deletion of Nrxn3, alternative splicing played no role in synaptic transmission in that brain region. My findings provide the first evidence that a single neurexin can have a distinct, non-overlapping function in a synapse-specific manner, which argues against the decades-old view that all neurexins and their gene products are functionally redundant. Moreover, I demonstrated that regulated alternative splicing does play a biologically significant role to transsynaptically regulate synaptic strength.

***Aoto J.*[#], Földy C, Ciurea-Ilcus SM, Tabuchi K, Südhof TC. (2015) Distinct Circuit-Dependent Essential Functions of Neurexin-3 in Regulating Presynaptic Release or Postsynaptic AMPA-Receptor Stability. *Nature Neuroscience.* 18(7): 997-1007. PMID: 26030848

[#]*Corresponding Author*

Chanda S, ***Aoto J***, Lee SJ, Wernig M, Südhof TC. Pathogenic Mechanism of an Autism-Associated Neuroligin Mutation Affects AMPA-Receptor Trafficking. *Molecular Psychiatry*. PMID: 25778475

Aoto J., Martinelli, DC, Tabuchi H, Malenka RC, Südhof TC. (2013) Presynaptic neurexin-3 alternative splicing trans-synaptically controls postsynaptic AMPA receptor trafficking. *Cell*, 154: 75-88. PMID: 23827676

4. In collaboration with Dr. Garret Anderson in the Südhof laboratory, we discovered that beta neurexins function redundantly to regulate tonic endocannabinoid tone at excitatory synapses in the hippocampus. These findings identified the first case of endocannabinoid usage at excitatory synapses in the hippocampus and provide insight into first molecular pathway that is involved in their regulation at these synapses. In contrast to my work on neurexin-3, this work suggests that in addition to unique functions, neurexin can function redundantly to regulate core synaptic properties.

Anderson GR, ***Aoto J,*** Tabuchi K, Földy C, Covy J, Yee AX, Wu D, Chen L, Malenka RC, Südhof TC. (2015) Presynaptic β-Neurexins Control Excitatory Synaptic Strength and Regulate Synaptic Endocannabinoid Signaling. *Cell.* 162(3): 593-606. PMID: 26213384

Complete List of Published Work in MyBibliography

http://www.ncbi.nlm.nih.gov/sites/myncbi/1X57Gihq5aX/bibliography/43450847/public/?sort=date&direction=ascending

D. Research Support

Ongoing Support

R00 MH103531-03 PI: Aoto 1/15/16-1/14/19

The major goals of this project are to continue the molecular, synaptic and cell-type dissection of the subiculum (from the K99) and how its dysfunction can result in neuropsychiatric disorders and addiction. The application of the K99 training will be heavily utilized to provide the first high-resolution characterization of projection circuits of the subiciulum.

Completed Support

K99 MH1035131 PI: Aoto 4/1/14-3/31/16

There are two major goals intended for this grant: 1. To receive training in cutting edge neuroscience techniques to functionally trace and analyze cell-type specific connectivity between the subiculum and downstream brain regions associated with schizophrenia and drug addiction. 2. To apply the training acquired to begin the experimental cell-type and synapse specific dissection of the subiculum and its downstream targets.

11POST7360078 PI: Aoto 7/1/11-6/30/13

The goal of this grant was to receive training in acute slice electrophysiology to characterize the synaptic function of neurexin-3.