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BIOGRAPHICAL SKETCH

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NAME: Smith, Stephen J

eRA COMMONS USER NAME (credential, e.g., agency login): SMITH.STEPHEN

POSITION TITLE: Senior Investigator, Allen Institute for Brain Science

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
Reed College, Portland Oregon	B.A.	06/1970	Psychology
University of Washington, Seattle WA	Ph.D.	06/1977	Physiology/Psychology
University of California, Berkeley	Postdoctoral	06/1980	Physiology

A. Personal Statement

During a long career in research and teaching, I have explored neural calcium signaling, synaptic vesicle dynamics, synaptogenesis, synaptic plasticity and neuromodulatory signaling synaptic network mechanisms through scholarship, experimental work and laboratory leadership. Most of this work has involved the development of novel computational imaging methods and their application to hard neuroscience and cell biology problems. My lab also had a very strong record of high-impact collaborative application of its novel imaging methodologies. We worked actively to disseminate our novel methods through both methodological and substantive publications, and teaching in numerous advanced courses at Stanford, Woods Hole, Cold Spring Harbor and other expert education venues. I led an NIH-funded consortium of neuroscientists and computer scientists aimed at disseminating knowledge, materials and tools to advance synaptomic applications of array tomography, a volumetric super-resolution light and electron microscopic imaging method we introduced in 2007. I moved in 2014 to the Allen Institutes, where I have enjoyed intense collaborations with outstanding genomic data science and computational neuroscience colleagues aimed at exploring that institutions' enormous bodies of transcriptomic and structural neuroscience data. Four publications I highlight here represent the introduction of array tomography from my earlier Stanford years (102,105) and my new directions from the Allen Institute years (144,146). I expect to return to Stanford campus life soon.

146. Liu YH, Smith SJ, Mihalas S, Shea-Brown E, Sümbül U. (2021) Cell-type-specific neuromodulation guides synaptic credit assignment in a spiking neural network. *Proc Natl Acad Sci U S A*. 21;118(51):e21118.

144. Smith SJ, Hawrylycz M, Rossier J, Sümbül U. (2020) New light on cortical neuropeptides and synaptic network plasticity. *Curr Opin Neurobiol*. 2020 Jul 14;63:176-188.

115. Micheva, K.D., Busse, B.L., Weiler, N.C., O'Rourke, N. and Smith, S.J (2010) Single-synapse analysis of a diverse synapse population: Proteomic imaging methods and markers. *Neuron* 68:639-653.

102. Micheva, K.D., and Smith, S.J (2007) Array tomography: A new tool for imaging the molecular architecture and ultrastructure of neural circuits. *Neuron* 55:25-36.

B. Positions and Honors

Positions and Employment

1981- 1984 Assistant Professor of Physiology, Yale Medical School
1984- 1987 Assistant Professor of Molecular Neurobiology, Yale Medical School
1986- 1987 Assistant Investigator, Howard Hughes Medical Institute
1987- 1989 Associate Professor of Molecular Neurobiology, Yale Medical School
1987- 1989 Associate Investigator Howard Hughes Medical Institute
1989- 1994 Associate Professor of Molecular & Cellular Physiology, Stanford University
1994- 2014 Professor of Molecular & Cellular Physiology, Stanford University
2014- Pres Professor Emeritus of Molecular & Cellular Physiology, Stanford University
2014- Pres Senior Investigator, Allen Institute for Brain Science, Seattle

Other Experience and Professional Memberships

1989- Pres NIH Study Sections, *ad hoc* and Chartered Service
1989- Pres NIH Site Visit Teams
1994- 2010 External Advisory Panel, *National Center for Microscopy & Imaging Research*, La Jolla
1994- 1995 Course Director, *Imaging Neuronal Structure & Function*, Cold Spring Harbor
1996- 1999 Director of Imaging Section, *Neurobiology*, Woods Hole
1999- 2004 Board of Scientific Counselors, National Institute of Child Health & Human Development
2000- 2004 Scientific Advisory Board, Max Planck Institute, Heidelberg, Germany
2000- 2014 Faculty, Imaging Section, *Neurobiology*, Woods Hole

C. Contribution to Science

The following paragraphs provide examples of the mixture of technology development and collaborative application efforts that has defined my personal path in neuroscience. In each of these cases mentioned here, this potent combination has resulted in major advances in our understanding of basic biological mechanisms and the opening of new fields to investigation.

1. Calcium Dynamics and Presynaptic Function

My doctoral dissertation work, supervised by Profs. Charles F. Stevens and Wolfhard Almers and completed in 1977, led me to an early recognition of the importance of intracellular free calcium dynamics to the regulation and temporal patterning of neuronal activity. For my postdoctoral work, I developed novel methods to measure intracellular Calcium and used these to test the computational model of calcium dynamics I developed as part of my doctoral thesis work. A gratifying correspondence of theory and experiment was the result (4). The Calcium diffusion/binding model introduced by this work was the first of the kind and has proven very influential, persisting as the backbone of most calcium dynamics theory to the present day. Upon gaining a faculty post, I further refined my Arsenazo III spectrometric Calcium-measurement method and applied it to make the first measurements of intracellular free calcium dynamics in a vertebrate neuron (9) and to make measurements of Calcium transients in the squid giant synapse that were critical in resolving long controversies about the dependence of neurotransmitter release on presynaptic Calcium and transmitter release (14). When Roger Tsien's superior fluorescence Calcium probes eventually became available, I adopted them early on to make further highly influential measurement of presynaptic Calcium that have influenced all subsequent thinking about the temporal and spatial structure of presynaptic Calcium transients (28).

28. Smith, S.J. and Augustine, G.J. (1988) Calcium ions, active zones and neurotransmitter release. *Trends Neurosci.* **11**:458-464.

14. Augustine, G.J., Charlton, M.P. and Smith, S.J. (1985) Calcium entry and transmitter release at voltage-clamped nerve terminals of squid. *J. Physiol.* (Lond) 367: 163-181.
9. Smith, S.J., MacDermott, A.B. and Weight, F.F. (1983) Detection of intracellular calcium transients in sympathetic neurones using arsenazo III. *Nature* **304**: 350-352.
4. Smith, S.J. and Zucker, R.S. (1980) Aequorin response facilitation and intracellular calcium accumulation in molluscan neurones. *J. Physiol.* (Lond) 300: 167-196.

2. Plasticity of Synaptic Structure and Function

My interests in synaptic calcium mechanisms and growing experience with optical physiology methods led me to explore other aspects of synapse development and function. One early result (16) proved of enormous lasting significance: the discovery that NMDA-type glutamate receptor channels are capable of directly allowing an influx of calcium ions. This long reverberating discovery was the result of a vibrant collaboration where my role was engineering intracellular calcium measurement. At the time (prior to Roger Tsien's introduction of suitable fluorescence probes), this was a difficult feat where I temporarily possessed a near monopoly. I recognized at the time the likelihood that such NMDA-receptor-channel calcium flux would be a key to understanding lasting forms of synaptic plasticity (24) – an expectation that has since been abundantly borne out. I also applied my growing expertise in optical methods and computational microscopy to influential explorations of neuronal cytoskeletal dynamics related to developmental axon and dendrite growth and to synaptic plasticity (29,85).

85. Niell, C.M., Meyer, M.P and Smith, S.J (2004) In vivo imaging of synapse formation on a growing dendritic arbor. *Nature Neurosci.* 7: 254-260.
29. Smith, S.J. (1988) Neuronal Cytomechanics: The actin-based motility of growth cones. *Science* 242: 708-715.
24. Smith, S.J. (1987) Progress on LTP at hippocampal synapses: A post-synaptic Ca trigger for memory storage? *Trends Neurosci.* 10: 142-44.
16. MacDermott, A.B., Mayer, M.L., Westbrook, G.L., Smith, S.J., and Barker, J.L. (1986) NMDA-receptor activation increases cytoplasmic calcium concentration in cultured spinal cord neurones. *Nature* 321: 519-522.

3. Astrocyte Signaling

Studies originally designed to explore excitotoxicity mechanisms in mixed cultures of hippocampal neurons and glial led in the late 1980s to an accidental but highly influential discovery of glutamate-triggered calcium signals capable of active propagation in "bystander" astrocytes. Since glutamate is the brain's predominant neurotransmitter and is certain to impact astrocytes *in situ*, this discovery quickly made it to the cover of Science magazine (33) and has reverberated strongly through the literature ever since. Follow-up work in our own laboratory demonstrated that synaptically released glutamate could trigger astrocyte calcium waves in hippocampal slices (40). Other work showed that glutamate application could induce strong dynamics of the astrocytic actin cytoskeleton (35). Two more recent collaborations made use of array tomography to further advance our knowledge of astrocyte roles in brain development and homeostasis (126,133).

133. Pașca, A.M., Sloan, S.A., Clarke, L.E., Tian, Y., Makinson, C.D., Huber, N., Kim, C-H., Park, J-Y., O'Rourke, N.A., Nguyen, K.D., Smith, S.J, Huguenard, J.R., Geschwind, D.H, Barres, B.A., and Pașca, S.P (2015) Generation of functional cortical neurons and astrocytes from human pluripotent stem cells in 3D cultures. *Nature Methods* 12(7):671-8. PMCID: PMC4489980.
126. Chung, W.S., Clarke, L.E., Wang, G.X., Stafford, B.K., Sher, A., Chakraborty, C., Joung, J., Foo, L.C., Thompson, A., Chen, C., Smith, S.J and Barres, B.A. (2013) Astrocytes mediate synapse elimination through the MEGF10 and MERTK phagocytic pathways, *Nature* 504: 394-400. PMCID: PMC3969024.

40. Dani, J.W., Chernjavsky, A, and Smith, S.J. (1992) Neuronal activity triggers Ca waves in hippocampal astrocyte networks. *Neuron*, 8: 429-440.
33. Cornell-Bell, A.H., Finkbeiner, S.M., Cooper, M.S. and Smith, S.J (1990) Glutamate induces calcium waves in cultured astrocytes: Long-range glial signalling. *Science* 247: 470-473.

4. Synaptic Vesicle Recycling

My laboratory was first to succeed in adapting Prof. William Betz FM 1-43 method (introduced using a frog neuromuscular junction preparation) to study vesicle release and turnover in mammalian CNS neurons (48). We disseminated this methods breakthrough aggressively and it opened major new avenues for the study of the basic mechanisms, diversity and plasticity of CNS presynaptic vesicle function (61,63). We also used the method to make the first measurements of single synaptic vesicle release events (65).

65. Ryan, T.A., Reuter, H. and Smith, S. J (1997) Optical detection of quantal presynaptic membrane turnover. *Nature* 388: 478-482.
63. Ryan, T.A., Li, L., Chin, L.-S., Greengard, P. and Smith, S.J (1996) Synaptic vesicle recycling in synapsin I knock-out mice. *J. Cell Biol.* 134: 1219-1227.
61. Ryan, T.A., Ziv, N.E. and Smith, S.J (1996) Potentiation of evoked vesicle turnover at individually resolved synaptic boutons. *Neuron* 17: 125-134.
48. Ryan, T.A., Reuter, H., Wendland, B., Schweizer, F.E., Tsien, R.W. and Smith, S.J. (1993) The kinetics of synaptic vesicle recycling measured at single presynaptic boutons. *Neuron*, 11, 713-724.

5. Synaptomics

As methods for functional and molecular analysis of unitary synapses, and for brain-wide and single-cell analysis of synaptic protein mRNA transcripts, have evolved in recent years, it has become increasingly clear that CNS synapses are highly diverse in protein composition, structure, function and plasticity (122). I introduced the term “synaptomics” to refer to experimental programs that focus on exploration of synapse diversity, driven by expectations that synapse diversity, and especially diversity of synaptic adhesion and modulatory signaling and plasticity mechanisms, will prove fundamental to understanding the development, function and plasticity of the brain’s synaptic networks. My laboratory has applied a variety of methods to explore synapse diversity over the years (71,76,78). Recently, this work has concentrated on applications of array tomography (as highlighted in section A Personal Statement above). In the work proposed by the present application, we’ll work to optimize, validate and disseminate new and improved array-tomography-compatible protein labeling reagents useful to further explore the origins and consequences of deep synapse diversity.

122. O’Rourke, N.A., Weiler, N.C., Micheva, K.D. and Smith, S.J (2012) Deep molecular diversity of mammalian synapses: Why it matters and how to measure it. *Nature Reviews Neuroscience* 13:365-79. PMCID: PMC3670986.
78. Waters, J. and Smith, S.J (2002) Vesicle pool partitioning influences presynaptic diversity and weighting in rat hippocampal synapses. *J. Physiol.,* 541(Pt 3):811-23.
76. Hopf, F.W., Waters, J., Mehta, S. and Smith, S.J (2002) Stability and plasticity of developing synapses in hippocampal neuronal cultures. J. Neurosci. 22(3):775-781.
71. Jontes, J.D. and Smith, S.J (2000) Filopodia, spines and the generation of synaptic diversity. *Neuron* 27, 11-14.