Research Statement

The goal of my research is to understand at the cellular and molecular level how synaptic connections form during development of neural circuits in the mammalian cortex. My approach to this problem combines time-lapse imaging to observe nascent synapse formation, and fluorescence recovery after photobleaching (FRAP) to measure protein dynamics, with molecular manipulations of synaptic proteins to decipher their roles in the growth of dendritic spines and synapses. In addition, I use physiological measurements in combination with two-photon uncaging of glutamate to examine the function of nascent synapses at the single synapse level.

Most of the excitatory synaptic connections in the cortex occur on dendritic spines, tiny protrusions that extend from the dendritic membrane. Dendritic spines are highly dynamic during development both in vitro and in vivo; periods of high motility coincide with synapse formation. Spine motility, driven by actin dynamics, is thought to allow the postsynaptic neuron to explore and sample presynaptic partners. In addition, alterations of spine dynamics and stability have been observed in response to sensory experience, and spine growth has been demonstrated in response to plasticity-inducing synaptic stimuli, leading to the hypothesis that these anatomical changes underlie the adaptive remodeling of cortical circuits. Thus, the identification and characterization of the molecules and mechanisms that control spine morphogenesis will be a crucial step toward understanding the formation and plasticity of cortical circuits.

To characterize changes in the spine actin cytoskeleton that promote spine growth and synaptogenesis, I used FRAP of GFP-actin to examine actin dynamics in dendritic spines, in combination with time-lapse two photon imaging to look at spine motility. By manipulating expression of the neuronal actin-binding protein, NrbI, and its mutants, I obtained evidence that regulation of the actin cytoskeleton to promote F-actin polymerization and increased spine motility can be sufficient to stimulate spine morphogenesis and synapse formation. Interestingly, those cells with extra synapses homeostatically downregulate individual response sizes to keep total synaptic input constant. These studies support a model in which the modulation of F-actin polymerization, in response to environmental factors or intrinsic developmental programs, controls spine motility rate, which in turn alters the rate of formation of new spines and synapses.

The ultimate test of a new synapse is an assay of its functionality. When do nascent spines acquire the ability to process synaptic signals? Is the nascent spine membrane already rich in glutamate receptors at the time of spine outgrowth, or do these accumulate after some time? Do all new spines form synapses? To examine the acquisition of glutamate receptor responses in nascent spines, I am presently combining time-lapse imaging to identify nascent spines with two-photon glutamate uncaging and physiology to measure responses to stimulation of single spines (see Figure 1, below).

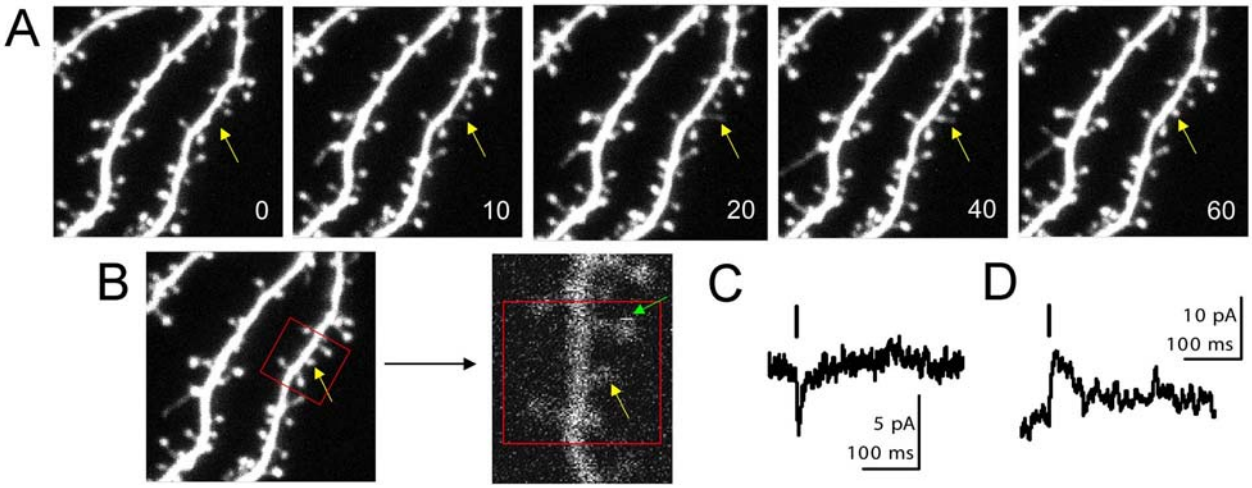

Figure 1. (A) Time-lapse images of dendrites from a CA1 pyramidal neuron transfected with GFP. Time stamps are in minutes. A new spine formed during the imaging session (yellow arrow). (B) Following time-lapse, currents were recorded from the same cell in response to glutamate uncaging (green arrow). (C, D) Responses to uncaging of glutamate at a single spine (vertical bar marks time of stimulus) at –65 mV (C) or at +40 mV (D).

Future Plans

In my own lab I intend to continue my search for the molecules and mechanisms that underlie spine morphogenesis and synapse formation. My research will combine the optical imaging and physiological tools that I learned as a postdoc with the molecular genetic tools that I applied as a graduate student. I will focus on the following projects.

Project#1: To probe the link between actin dynamics and spine morphogenesis. My previous work on spine actin dynamics supports the hypothesis that F-actin polymerization and increased spine motility lead to spine morphogenesis and synapse formation. Conversely, does reduced actin polymerization and/or less motile spines lead to reduced number of spines and fewer synaptic contacts? Many regulators of the actin cytoskeleton have been identified in dendritic spines. In fact, one such regulator, profilin, has been shown to reduce spine motility when targeted to the spine head. Using FRAP of GFPactin to examine the effects of these regulators of the actin cytoskeleton on spine actin dynamics, and time-lapse imaging to examine spine motility, in combination with quantification of spine and synapse densities, I will further pursue the relationship between actin polymerization, spine motility, and synapse formation.

Project#2: To identify molecules that anchor and stabilize synaptic proteins. The formation of new synapses requires the assembly of a large number of pre- and postsynaptic proteins at new synaptic sites, as well as their precise alignment across the synaptic cleft. How are synaptic proteins directed to and stabilized at nascent synapses? This could occur via targeted delivery and/or by selective stabilization at synaptic sites. My work on NrbI localization demonstrated that NrbI actin binding is responsible for its accumulation in dendritic spines. However, FRAP of GFPNrbI identified an additional sequence element of fifteen amino acids that further stabilizes NrbI in dendritic spines. None of the known interactions of NrbI (actin, protein phosphatase I,

p70S6K, kalirin-7, TGN38, or NrbII) map to this site. Using the yeast two-hybrid assay with the N-terminal domain of NrbI, with and without the fifteen amino acid stabilizing element, I will identify the protein(s) that stabilize NrbI in spines. These experiments will provide important information about the organization of the postsynaptic scaffold, and potentially could lead to the identification of additional members of the postsynaptic specialization.

Project#3: To identify the molecules responsible for initiation of spine morphogenesis. Elucidating the molecular mechanisms of synapse formation requires not only knowledge of the identity of those proteins that comprise the mature synapse, but also information about the time at which they arrive at sites of new synapse formation. Those molecules that play a role in the initiation of synapse formation and in the initial assembly of pre- and postsynaptic specializations are expected to arrive in the early stages of synaptogenesis. Using dual color time-lapse imaging, I will simultaneously observe the formation of nascent spines and the accumulation of GFP-tagged synaptic proteins. My previous work implied a role for NrbI in the induction of dendritic spines. Other sources have identified NrbII as a negative regulator of spine morphogenesis. I will begin by examining the temporal dynamics of NrbI and NrbII recruitment to nascent spines. In addition to monitoring the arrival of proteins at the synapse optically, molecules that affect synaptic response properties, such as channels and receptors, will be monitored physiologically, using two-photon uncaging.

Project#4: To identify determinants of stability of nascent spines. Spines exhibit different lifetimes: some are transient, living only minutes, others can survive for days or even months, perhaps years. What determines the stability of nascent spines? One hypothesis is that spines with stronger synapses are more stable. I am developing chronic imaging methods in which spines can be repeatedly observed over the course of days or even weeks. Spines of different lifetimes will be tested and compared to determine whether there is any systematic variation in response amplitude with spine lifetime. A second hypothesis is that synaptic activity plays a role in spine stabilization. To test this, I will use time-lapse imaging to identify nascent spines in combination with two-photon glutamate uncaging at different frequencies to investigate whether frequency or total amount of glutamate stimulation can affect spine lifetime. In addition, simultaneous dual color imaging of nascent spine formation and synaptic protein distribution promises to identify those proteins whose arrivals are correlated with enhanced spine stability. Roles for candidate molecules will be tested using RNAi to knockdown protein levels and subsequent examination of spine stability.

The proposed experiments promise to increase our basic knowledge about the cellular and molecular mechanisms of synapse formation. Specifically, I will investigate the signaling events responsible for morphogenesis and stabilization of dendritic spines during the formation of synapses in the cortex. My experiments are directed at a very specific subset of synapses, but they also should provide information on the general mechanisms of spine morphogenesis and synapse formation in the mammalian cortex. Notably, many neurological diseases resulting in mental retardation have been associated with spine loss or spine morphology changes. It is through basic knowledge of the molecules and mechanisms in developmental signaling systems that we can begin to shed light on the diverse mechanisms that contribute to human disease.

Prior research experience

Ever since the summer of 1990 that I spent studying transcriptional control as an undergraduate in the laboratory of Winship Herr at Cold Spring Harbor Laboratory, I have known that I would like to pursue a research career in biology. I enjoyed the thought process that went into designing experiments, and I liked the feel of being at the bench. If there were any doubts remaining when I returned to Indiana University, they were gone after meeting Norm Pace, who was truly an inspiration to me scientifically. In the Pace laboratory, I studied the structure and function of an RNA enzyme, RNaseP.

My fascination with synapse formation began as a graduate student in the laboratories of Corey Goodman and Ehud Isacoff at the University of California, Berkeley. There, I used molecular and genetic techniques to study synapse formation at the neuromuscular junction (NMJ) of the fruit fly, *Drosophila melanogaster*. First, I identified sequences necessary and sufficient for clustering molecules to the synapse. Then, I used time-lapse imaging to examine the development of the NMJ in living transgenic larvae that expressed a synaptically targeted green fluorescent protein (GFP) marker. By combining in vivo time-lapse imaging with ultrastructural analysis, we were able to capture the first convincing glimpses of nascent synapses in a living animal.

Prior teaching experience

My passion for research, however, is not exclusive, in that I have always also enjoyed teaching. In high school I spent three mornings every week volunteering as an assistant music director at the local junior high school. When I was an undergraduate math major, I worked as an intern in the math department, assisting with a calculus course for non-science majors. In graduate school, I was a teaching assistant for two semesters. Both were introductory courses for upper level biology majors- genetics and neurobiology. I enjoyed having the opportunity to teach, and the students responded by voting me Outstanding Graduate Student Instructor. During my travels following graduate school, I had a wonderful opportunity to teach part of a developmental neurobiology course at the TATA Institute in Bangalore, India. These experiences reconfirmed my enthusiasm and commitment to include teaching as part of my long-term career goals.