**Cellular and molecular mechanisms of associative and non-associative learning**

I am currently combining behavioral, cellular, and molecular biological approaches to delineate the changes that underlie simple forms of learning and memory in invertebrates. My research focuses on the siphon-withdrawal reflex of *Aplysia*. I have been studying associative and non-associative forms of learning: sensitization, habituation, dishabituation, and classical conditioning. A key to understanding the mechanisms of behavior lies in investigating plasticity at specific synapses in the CNS. The *Aplysia* siphon-withdrawal reflex has been advantageous for such studies. The reflex undergoes associative and non-associative learning with many of the behavioral features of learning in mammals, suggesting that associative and non-associative learning in *Aplysia* and mammals may share common mechanisms.

I use electrophysiological, biochemical, and imaging techniques to investigate cellular mechanisms contributing to associative and non-associative learning.
I showed that monosynaptic connections from siphon sensory neurons to siphon motor neurons make a substantial contribution to the reflex in simplified preparation of *Aplysia* (Antonov et al., J Neurosci 1999 Dec 1;19(23):10438-50.). I then assessed the contribution of various cellular mechanisms to classical conditioning of the reflex and found that it is due in part to associative activity-dependent plasticity at synapses from the sensory neurons to motor neurons (Antonov et al., J Neurosci 2001 Aug 15;21(16):6413-22). I next investigated the possible contribution to the conditioning of two associative cellular mechanisms: activity-dependent neuromodulation involving enhanced activation of the PKA pathway in the sensory neuron, or Hebbian long-term potentiation (LTP) involving enhanced Ca2+ elevation in the motor neuron. My results provide the most direct evidence to date that each of these mechanisms contributes to behavioral learning, and also suggest that the two mechanisms are not independent but rather interact through retrograde signaling (Antonov et al., Neuron, Vol 37, 135-147, January 2003.).

I now propose to extend these studies in several ways.

First, I will perform additional experiments to test the roles of these synaptic mechanisms in behavior. In the experiments I have performed so far, injecting the Ca++ chelator BAPTA into a single LFS neuron or the PKA inhibitor PKAi into a single LE neuron has blocked plasticity of the monosynaptic PSP but has not blocked behavioral learning, presumably because the behavioral stimuli activate several LE and LFS neurons in parallel. I will therefore test whether injecting two or three LE or LFS neurons in the same preparation reduces behavioral conditioning.

Second, I will examine pre- and postsynaptic molecular mechanisms of the plasticity during conditioning. Recent studies of LTP in mammalian systems indicate that it involves insertion of AMPA-type glutamate receptors into the postsynaptic membrane. I have found that the monosynaptic PSP in Aplysia also has an AMPA component, and I will test whether conditioning involves insertion of new AMPA receptors. In addition, I will examine retrograde signaling and possible presynaptic mechanisms. My colleagues and I have found that bathing the abdominal ganglion in the nitric oxide (NO) synthase inhibitor nitroarginine blocks behavioral conditioning, suggesting that NO could be a

retrograde messenger. I will test this idea at the cellular level, and I will also examine possible presynaptic targets of NO including PKG and ryanodine-sensitive Ca2+ stores. These studies should begin to elucidate coordinate pre- and postsynaptic mechanisms of plasticity that contribute to behavioral learning in *Aplysia*.

Third I will perform imaging studies to investigate structural changes during associative and non-associative learning. The early phase of synaptic plasticity in many systems is accompanied by the rapid redistribution of synaptic proteins. Facilitation by serotonin in Aplysia and synaptic activity in hippocampal neurons are both accompanied by a decrease in the number of clusters or puncta of the synaptic vesicle associated protein synapsin, which is thought to reflect vesicle mobilization. On the other hand, the onset of long-term potentiation in hippocampal neurons is accompanied by a rapid increase in the number of puncta of synapsin and another vesicle associated protein, synaptophysin, as well as the GluR1 subunit of postsynaptic AMPA receptors and sites where the pre- and postsynaptic proteins co-localize. These changes are thought to reflect either the rapid formation of new functional synapses or an early step in that process. I have begun to perform experiments to examine the possible redistribution of synaptic proteins during conditioning, first focusing on the presynaptic protein synaptophysin. My colleagues and I have cloned a DNA construct for a synaptophysin-GFP (Green Fluorescent Protein) fusion protein into the Aplysia expression vector pNEX3 and microinjected the purified plasmid DNA into the sensory neuron. Expression of the fluorescent protein in living neurons can be visualized one day later on a 2-photon microscope.