

### 3D TIME-LAPSE IMAGING OF HIPPOCAMPAL DENDRITIC SPINE PLASTICITY USING 2-PHOTON MICROSCOPY

Steve M. Potter\*, David B. Kantor, Adam N. Mamelak, Scott E. Fraser, and Erin M. Schuman. Caltech Div. of Biology, Pasadena, CA 91125

Numerous studies have established that neurotrophins can promote profound morphological changes during neuronal development. Our recent work in area CA1 of the adult rat hippocampal slice has shown that BDNF and NT-3 can dramatically enhance synaptic transmission, and this enhancement depends on protein synthesis within the dendrites. Given that mRNAs for cytoskeletal proteins and protein synthesis machinery are present in or near dendritic spines, the question arises as to whether BDNF and NT-3 can produce morphological changes in synaptic structures that are visible by light microscopy.

Most attempts to correlate synaptic potentiation with morphological changes have utilized fixed specimens, visualized by EM or light microscopy. To investigate the dynamics of potential morphological changes, we developed a 2-photon system for imaging dendritic spines in living hippocampal slices. The greatly reduced phototoxicity and photobleaching, and enhanced signal/noise afforded by two-photon laser-scanning microscopy (TPLSM) allowed us to image three-dimensional structures with submicron resolution, repeatedly for over 12 h. Slice health was verified electrophysiologically before and after imaging, including the ability to exhibit LTP. In preliminary experiments, we have observed rapid (<30 min) changes in spine length and orientation in neurons labeled extracellularly with the membrane dye, DiO, or intracellularly with fluoresceindextran. We will examine how these spine dynamics are affected by exposure to neurotrophins.

Supported by NIH Fellowship NS 10257-01 and the Beckman Foundation (SMP), and Amgen (EMS)