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MEASUREMENT OF CALCIUM TRANSIENTS IN PYRAMIDAL NEURONS OF HIPPOCAMPAL SLICES VISUALIZED BY CONFOCAL MICROSCOPY.

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Central to models of long-term potentiation is the role of postsynaptic elevations in calcium ion concentration in mediating synaptic modifications. The major path for this influx is thought to be through the NMDA receptor. Calcium influx through voltage-dependent calcium channels may also significantly contribute to the total cytoplasmic elevation. Initiation of various calcium-activated processes are then thought to give rise to an increase in synaptic efficacy.

We are investigating changes in intracellular calcium concentration in pyramidal neurons of hippocampal slices *in vitro*. Individual neurons are impaled using a glass microelectrode filled with the fluorescent calcium indicator fluo-3. A cell may then be filled with the dye by iontophoresis and followed physiologically during the course of the experiment. Fluorescent signals are monitored using a Bio-Rad MRC-600 laser scanning confocal microscope. Fluorescent measurements are made at high spatial resolution, with < 1 um depth of field. Individual scan lines may be taken at 2msec intervals to provide a fine dynamic record of the calcium changes within the cell. Electrical and calcium signals are recorded from cells activated either synaptically by axons stimulated with bipolar electrodes in the stratum radiatum or by depolarization by direct current injection through the intracellular electrode. Sustained elevations in calcium are seen in the basal and apical dendrites and the soma following high frequency stimulation under conditions that induce LTP.