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SPATIAL AND TEMPORAL RESOLUTION OF CALCIUM TRANSIENTS PRODUCED BY STIMULATION OF DIFFERENTIATING AMPHIBIAN SPINAL NEURONS.

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Action potentials evoked from *Xenopus* spinal neurons exhibit a calcium dependence at initial stages of differentiation that shifts to a sodium dependence over a 24 hr period (Spitzer and Lamborghini, 1976). Therefore, stimulation is expected to produce greater calcium influx at early stages of differentiation than during later periods of development. However, the intracellular free calcium level attained upon stimulation is likely to be influenced by other factors such as the release of calcium from intracellular stores and calcium sequestration and buffering. These variables may also change over development.

Changes in the intracellular calcium levels produced by depolarization have been examined in intact (non-dialyzed) cultured neurons using the calcium indicator, fluo-3 and high speed confocal image analysis. Elevations of intracellular free calcium in response to depolarization occur throughout the cell, most notably in the nucleus. The initial onset of increased indicator fluorescence is not detectably delayed in the nucleus relative to the cytosol under measurement conditions with a time resolution as fine as 2 msec. The fluorescence increases to peak values within approx. 200 msec. The initial fluorescence decay was fitted to first order kinetics with a rate constant of about -0.15 sec^{-1}. After stimulation-evoked increases began to decline, slower elevations in fluorescence occurred. The contributions of calcium release from intracellular stores and calcium buffering to observed changes in intracellular calcium are considered. The effects of neuronal maturation on patterns of stimulus-evoked fluorescence will be described.

Supported by grants from the NIH to JH and NCS and from the NSF to TJS.