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FLUORESCENT MITOCHONDRIAL STAINS AS VITAL PROBES OF METABOLIC ACTIVITY IN THE RAT HIPPOCAMPAL SLICE PREPARATION. <u>S. R. Quartz<sup>2</sup>, R. J. Adams and T. J. Sejnowski<sup>2</sup></u>, Salk Institute, La Jolla, CA 92037 and <sup>2</sup>UC, San Diego, La Jolla, CA 92039.

Distribution of the cationic mitochondrial fluorescent dyes rhodamine 123 and 2-(4dimethylaminostyryl)-N-methylpyridinium iodide is governed primarily by the Nernst equation. Since mitochondrial membrane potentials have been reported to be -150mV in situ (Farkas et al., Biophys. J. 56, 1989), these dyes serve as vital stains of mitochondria. We have utilized these properties of the dyes as probes of metabolic activity in conjunction with confocal microscopy. In agreement with the histochemical localization of cytochrome oxidase (CO) (Kageyama & Wong-Riley, Neuroscience 7, 1982), we observe the following: 1) low to moderate staining in principal cell layers; 2) low staining of proximal apical dendrites of CA1 pyramidal cells; 3) intense staining of outer molecular layer, dentate gyrus; 4) intense staining of stratum oriens, CA3; 5) low average staining of stratum lucidum, CA3, under low magnification. However, under higher magnification we observe intensely stained mossy fibers and their synaptic extensions, suggesting that average intensity under low magnification may not be an accurate indication of activity. In contrast to CO localization, we have not found general intense staining in stratum moleculare (SM) of CA1, although, in agreement with those studies, we have observed highly reactive dendritic processes, presumably of interneurons, extending throughout the region. The lack of general staining in SM may be due to the finding that synaptic mitochondria are less active than nonsynaptic mitochondria (Leong et al., J. Neurochem, 42, 1984).

Recent studies report a 50% higher ATP content in cultured astrocytes than in cortical neurons (Hertz et al., <u>Neurochem, Res.</u> 13, 1988). In agreement with this finding, we observe intense staining in both protoplasmic and fibrous astrocytes. We have determined that these cells are astrocytes according to general morphological characteristics, distribution patterns, and by double-labelling with immunofluroescent markers for GFAP. These results suggests that these dyes may provide a simple *in vitro* assay for the study of astrocyte function.