

[<sup>3</sup>H]-2-DEOXYGLUCOSE UPTAKE IN THE MOLLUSCAN CENTRAL NERVOUS SYSTEM. Stephen C. Reingold & Terrence J. Sejnowski<sup>+</sup>. Department of Biology, Princeton University, Princeton, New Jersey 08544.

We have used [<sup>3</sup>H]-2-Deoxyglucose ([<sup>3</sup>H]-2-DG) to localize metabolically active neurons within the buccal ganglion of the mollusk Limax maximus. Autoradiographic analysis gives a resolution of 0.5  $\mu$ m, with label concentrated in cell somata and neuropil processes. Within somata, nuclei appear less heavily labelled than cytoplasm. Labelling of neurons may be correlated with intracellular stimulation and spontaneous activity within the ganglion.

Individual buccal ganglia were dissected and incubated in high Mg<sup>++</sup>, low Ca<sup>++</sup> saline to reduce transmission at chemical synapses. Peripheral nerve roots were recorded with suction electrodes. Glass microelectrodes were used to impale individual neurons within the ganglion. Upon impaling a neuron, the bath solution was replaced by 100-200  $\mu$ Ci [<sup>3</sup>H]-2-DG/ml high Mg<sup>++</sup> low Ca<sup>++</sup> saline. The cell was depolarized with intracellular current pulses to fire at a rate of 1-2 spikes/second, while monitoring peripheral nervous system activity. After 30-60 minutes of intracellular stimulation, the microelectrode was removed and the ganglion washed in 5 changes of non-radioactive saline over 30 minutes. Individual ganglia were freeze-substituted in acetone at -70°C or dehydrated in dry acetone at 4°C. Ganglia were embedded in Araldite or Spurr's Medium and serially sectioned at 3-5  $\mu$ m. Sections on slides were dipped in Kodak Nuclear Emulsion (NTE-2), exposed for 2-4 weeks, and subsequently developed and counter-stained.

In control experiments in which a neuron is impaled but not driven and peripheral activity is absent, several somata are often labelled. Rarely, a single cell was activated with current injection in the absence of spontaneous activity, and a single cell soma was labelled with [<sup>3</sup>H]-2-DG. In most experiments, several cells were labelled, even though only a single cell was experimentally activated. Such multiple cell labelling may result from spontaneous firing of normal or autoactive neurons; from activation of follower cells by the impaled neuron through electrical or still-functioning chemical synapses; or from sub-threshold activity or non-electrical metabolic activity.

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<sup>+</sup>Present address: Department of Neurobiology, Harvard Medical School, Boston, Massachusetts 02115.