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13. I have repeated Dayton's (*10*) demonstration of a limited dispersal distance of spores released from an attached sporophyte. When the adult plants exist as a point source, dispersal is limited to acceptable sites within a radius of 1.5 m.
14. I have "transplanted" *Postelsia* by cutting off the distal third of sporogenic plants, securing six in a hardware cloth mesh, and attaching this to rock substratum at least 0.3 m², freshly scraped clean of mussels or other epifauna or flora. Immature sporophytes appeared in five-sixths of the treatments in September 1975 at Tatoosh, and 14/14 initiated in September 1977. Two of the former populations are still in existence (August 1978) as are all of the latter.
15. The average rate of encroachment of *Mytilus californianus* was determined by measuring rate of movement into small ($\leq$ 100 cm², N = 44), intermediate ($<$ 3500 cm², N = 78), and large ($>$ 3500 cm², N = 11) experimental sites.
16. Corallinaceous algae generally typify areas subject to intense grazing pressure [R. T. Paine and R. L. Vadas, *Limnol. Oceanogr.* **14**, 710 (1969); P. J. Vine, *Mar. Biol.* **24**, 131 (1974)].
17. I thank M. Slatkin, J. F. Quinn, and P. D. Boersma for constructive advice, the U.S. Coast Guard for permission to conduct research on Tatoosh. Supported by NSF grant OCE 77-26901.

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2-Deoxy-D-Glucose Maps Movement-Specific Nervous Activity in the Second Visual Ganglion of *Drosophila*

Abstract. *Adult* Drosophila *were fed with tritium-labeled deoxyglucose prior to a 5-hour period of visual stimulation. A flickering disk of light and a moving grating were presented to the left and right eyes, respectively. Autoradiography revealed enhanced labeling solely in that part of the second optic ganglion (medulla) whose visual field was stimulated by movement.*

2-Deoxy-D-glucose (DG) is believed to be transported to and taken up by nerve cells through the same mechanisms as its structural analog, glucose, which is the major fuel for nervous tissue. Nerve cells with high physiological activity require rapid adenosine triphosphate synthesis to restore electrochemical gradients and take up more glucose, or, when offered, labeled deoxyglucose. After phosphorylation, the first step of glycolysis, DG-phosphate is not metabolized further and thus tends to accumulate preferentially in physiologically active cells.

Although this method of "activity staining," developed by Sokoloff and his colleagues (*1*), has not yet, to our knowledge, been demonstrated to be effective at the level of individual neurons, it has been successfully applied by several scientists to map nervous activity in various areas of the vertebrate brain under various conditions of stimulation (*2*). In a series of qualitative experiments we found that the method is effective in an invertebrate nervous system and can provide functional information in *Drosophila* yet inaccessible by electrophysiological techniques (*3*).

A 3- to 7-day-old female *Drosophila* that had been starved in an empty vial at 19° to 21°C for 24 to 36 hours was etherized briefly in order to be attached to a small brass rod which could be mounted either in the stimulus set-up or, by a special clamp, in a cryostat microtome (South London Electrical Equipment). The fly's head was immobilized by cementing it to the body. The flicker stimulus was generated on the left eye and the movement stimulus on the right eye (Fig. 1). The patterns were presented to the fly through two microscope objectives. This optical procedure (*4*, *5*) effectively prevents stray light from reaching receptors that are not directly stimulated.

At the beginning and halfway through the 5-hour stimulation period, the fly was offered a drop of 0.3 m*M* aqueous solution of tritiated DG (*6*) from the tip of a syringe and was allowed to drink to satiation. In this way each fly (weight, 1 mg) took up about 2 to 5 μCi of label. Although the precise concentration of label in the hemolymph was not known it certainly was higher by two to three orders of magnitude than that in similar experiments on vertebrates (*2*). At the end of the stimulation period, fly and holder were, under red light, immersed in a drop of embedding medium (OCT) and quickly frozen in melting nitrogen ("slush"). At −25°C, 12-μm-thick sections were cut with a knife cooled by acetone-CO_2 ice and picked up on −25°C slides. These were transferred to a freeze-dryer (Balzers BA-3) and kept below −50°C at 10^{-4} to 10^{-3} torr for 2 hours. The dry sections were carefully inspected, and any protruding elements like chitin, cement, or doubly layered pieces of tissue were removed before they were brought into close contact with slides coated with stripping film (Kodak AR-10). The sandwich of section and film between the two slides was clamped together with pressure at about

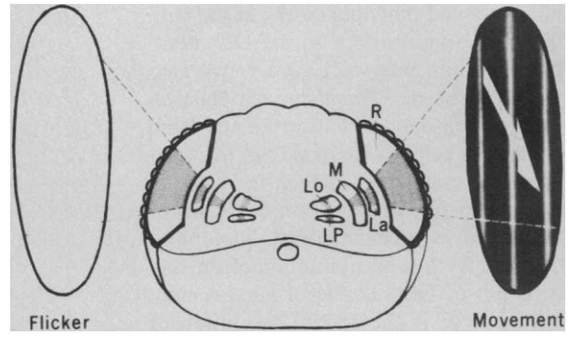

Fig. 1. Visual stimulus. The right eye was exposed to a moving sine-wave grating (angular diameter, 70°; mean luminance, $\bar{I}$ = 1500 cd/m²; pattern contrast, $\Delta I/\bar{I}$ = 0.40; spatial period, 20°; constant speed, 26° sec^{-1} from anterior to posterior). The left eye was exposed to a homogeneous disk of temporally modulated light. Luminance and sinusoidal modulation (depth and frequency) of the flicker stimulus were adjusted so that the light signals received by individual receptors on either eye were similar. (On the right eye, however, movement induced phase differences in the signals to neighboring receptors.) In the schematic horizontal cut through a *Drosophila* head, the stimulated portions of the retina (*R*) and the optic ganglia lamina (*La*), medulla (*M*), lobula (*Lo*), and lobula plate (*LP*) are stippled.

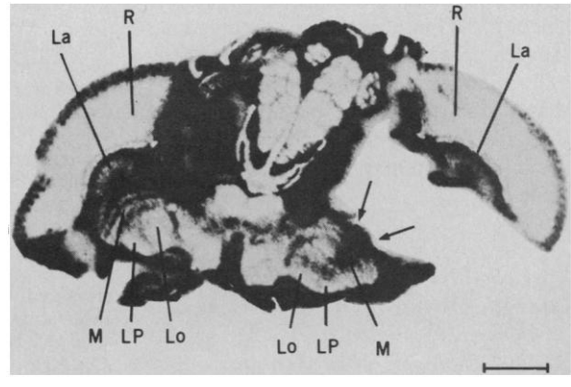

Fig. 2. Autoradiograph of a nearly horizontal section through the head of a *Drosophila* fed with [^{3}H]deoxyglucose. Hemolymph spaces are heavily labeled. The retina and optic ganglia are recognizable (compare with Fig. 1). The movement-stimulated part of the right medulla is strongly labeled (arrows). Scale marker, 100 μm. Note for comparison that one ocular dominance hypercolumn of the monkey visual cortex [770 μm (*2*)] would cover the entire section.

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200 g/cm² and kept at +4°C for 4 days prior to development.

In a typical autoradiograph (Fig. 2) one can easily identify in each hemisphere retina (*R*) and lamina (*La*) (displaced on right half), medulla (*M*), lobula (*Lo*), and lobula plate (*LP*). [For an anatomical review of the dipteran brain see (*7*).] In the medulla of the right eye, the portion stimulated by the moving grating was heavily labeled, whereas the left medulla displayed a comparatively homogeneous labeling of the various layers throughout flicker-stimulated and nonstimulated portions. No clear differences could be detected between stimulated and nonstimulated regions in retina and lamina on either side.

This distribution of radioactive label qualitatively prevails in six sections of the animal of Fig. 2; it was also visible in four sections of another animal treated identically and in five sections from two animals stimulated for 14 hours. Only these four flies have been subjected to the procedure so far. Autoradiographs of unlabeled material showed negligible traces of chemography and pressure artifacts. We interpret our results as indicating that (i) the DG method may be successfully applied to insect nervous systems, (ii) there is distinct movement-specific activity in the medulla of *Drosophila*, and (iii) nonspiking cells such as receptors and monopolar cells in the lamina may be refractory to the DG method as we currently carry it out. We suggest that the movement-specific label in the medulla may perhaps originate from columnar cells corresponding to "elementary movement detectors," which have been hypothesized on the basis of behavioral experiments with *Drosophila* (*5*). Any further interpretation of the distribution of label in Fig. 2 must remain speculative. If the spatial resolution of about 5 to 10 μm obtained with the technique can be improved so the amount of label in individual cells may be quantitatively evaluated (*8*), it should be possible to clarify whether active brain regions are labeled as a result of enrichment of DG in glial cells or extracellular spaces or whether active neurons themselves show preferential uptake. In the latter case the method may prove useful for investigating the circuitry of identified neurons in invertebrate nervous systems.

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Cell-Free Nitrogenase and Hydrogenase from Actinorhizal Root Nodules

Abstract. *Field-grown alder (*Alnus glutinosa*) root nodules were disrupted in liquid nitrogen to release the actinomycete endophytes. The endophytes were broken by mild sonic oscillation and yielded a cell-free nitrogenase preparation capable of reducing acetylene and protons. In addition, the preparation carried a cell-free uptake hydrogenase.*

Certain nonleguminous plants form perennial nitrogen-fixing root nodules in symbiosis with actinomycetes (*1*). This association was designated actinorhizal at a recent symposium, held at Harvard Forest, Petersham, Massachusetts, on actinomycete-nodulated plants. Such nodulated plants are often pioneers in nutrient-poor soils and participate in soil enrichment early in some ecological successions (*2*). They serve in forest restoration or land reclamation that requires long-term nitrogen input (*2*). Recent isolation of the nitrogen-fixing prokaryotic symbiont and confirmation that it is an actinomycete (*3*) have stimulated a resurgence of interest in the actinorhizal nodules.

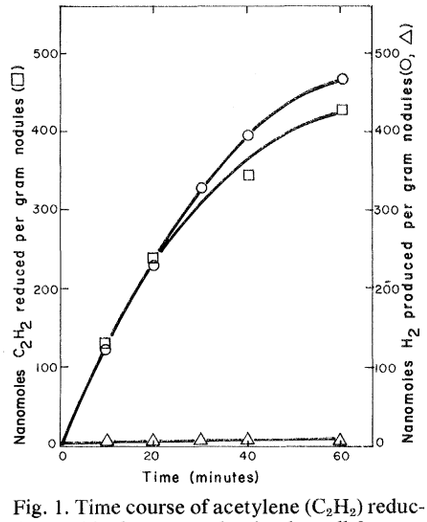

Fig. 1. Time course of acetylene (C_2H_2) reduction and hydrogen production by cell-free extracts from *Alnus glutinosa* root nodules. The assays were performed in 21-ml vaccine bottles at 30°C. The reaction mixture contained the same concentration of reactants as indicated in Table 1. The reaction was initiated by the addition of 1 ml of crude extract to give the reaction mixture a total volume of 2 ml. The gas phase consisted of 10 percent acetylene in argon for C_2H_2 reduction assays; C_2H_2 was omitted when hydrogen production was measured. Hydrogen was measured by gas chromatography with a thermal conductivity detector. Hydrogen production: ATP added (○); ATP omitted (△); C_2H_2 reduction (□).

There has been little biochemical study of the nodules, primarily because of the difficulties inherent in working with woody tissue containing an abundance of reactive phenolic substances that inactivate enzymes when cells are broken (*4*). Methods have been described for preparing particulate acetylene-reducing homogenates from actinorhizal nodules (*5*, *6*); however, there are no reports that enzymatically active cell-free extracts have been recovered. We report a technique for preparation of active cell-free nitrogenase and of an "uptake hydrogenase" from field-grown nodules of the European alder *Alnus glutinosa* L. Gaertn. The requirements for nitrogenase activity are similar to those for other nitrogen-fixing systems, again illustrating the highly conserved nature of the nitrogenase system among widely diverse prokaryotes.

Alnus glutinosa root nodules were collected from the University of Wisconsin Arboretum during the fall of 1978. Excised nodules were washed in 100 m*M* phosphate buffer containing 20 m*M* ascorbate at *p*H 7.0, and were stored frozen in liquid nitrogen. To release the endophyte, nodule samples (usually 5 g) were crushed to a fine powder in liquid

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