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THE EFFECT OF MONOCULAR EYE PATCHING ON FAST TRANSPORT IN THE TADPOLE OPTIC NERVE

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Sensory deprivation dramatically affects the anatomical and neurophysiological development of synapses. Even though such changes must be accompanied by biochemical ones there is little evidence linking any biochemical mechanism specifically to the changes associated with deprivation.

Since axons and terminals are virtually devoid of the cellular machinery for protein synthesis, proteins and glycoproteins destined for the synaptic region are synthesized in the cell body and transported down its axon. These experiments were designed to test the effects of light and form deprivation at an early stage of development on transport of newly synthesized protein from the ganglion cell bodies in the eye, down their axons in the optic nerve, to their synaptic terminals in the contralateral optic tectum. There are two groups of transported proteins, one fast (70 mm/day) and one slow (1 mm/day). The present study involved fast transport.

Tadpoles in the hind limb stage (tail/leg length ≃ 12) were anesthetized with MS222 and the right eye was injected with 3μCi (in 3 μl) of (^{3}H) proline or (^{3}H) fucose. In the experimental group a patch of skin taken from a frog was then sutured over the right eye. Tadpoles were isolated in separate chambers all connected to one central aerated tank maintained at room temperature. At variable intervals after injection the tadpoles were decapitated. Four controls and four deprived tadpoles were injected for each time point. The skulls and eyes were opened and placed in a 10 percent formalin solution for 24 hours. The optic tecta and retinas were dissected out, air dried, dissolved in 0.5 ml Solvene (Packard) and immersed in 10 cc of scintillation fluid (Packard) for scintillation counting. (^{3}H) proline labels proteins and glycoproteins and travels with both slow and fast components; (^{3}H) fucose labels glycoproteins and is considered to be a specific label for fast transported materials.

Since the retinal ganglion cells in the right eye project exclusively to the left tectum, radioactivity in the right tectum represents background or material delivered via the blood. Transported radioactivity (dpm) is therefore expressed as ($dpm_{\text{left tectum}} - dpm_{\text{right tectum}}$). In Figure 1 each point represents the mean of at least three tadpoles. In the control using (^{3}H) proline, transported proteins appeared in the left tectum at four hours with a peak amount present at 18 hours. In the test tadpoles (right-eye deprived) the mean amount accumulating in the left tectum was less at every time point until 24 hours. Statistical analysis indicated that the results were significant at four hours ($p<0.001$), eight hours ($p<0.02$), and 18 hours ($p<0.06$) and not significant at 12 and 24 hours. Almost identical results were obtained with (^{3}H) fucose as shown in Figure 2 but due to technical difficulties there were not enough animals for similar statistical analysis of the fucose-labelled transport. The effect was more pronounced in one group of older (four limb stage) tadpoles (tail/hind leg length ≃ 1). Twenty-four hours after injection the control mean was 3523 dpm and the deprived mean was 146 dpm (significant, $p<0.005$).

The decrease in accumulation might be ascribed to decreased incorporation of label rather than decreased transport. However previous investigators have found that deprivation does not

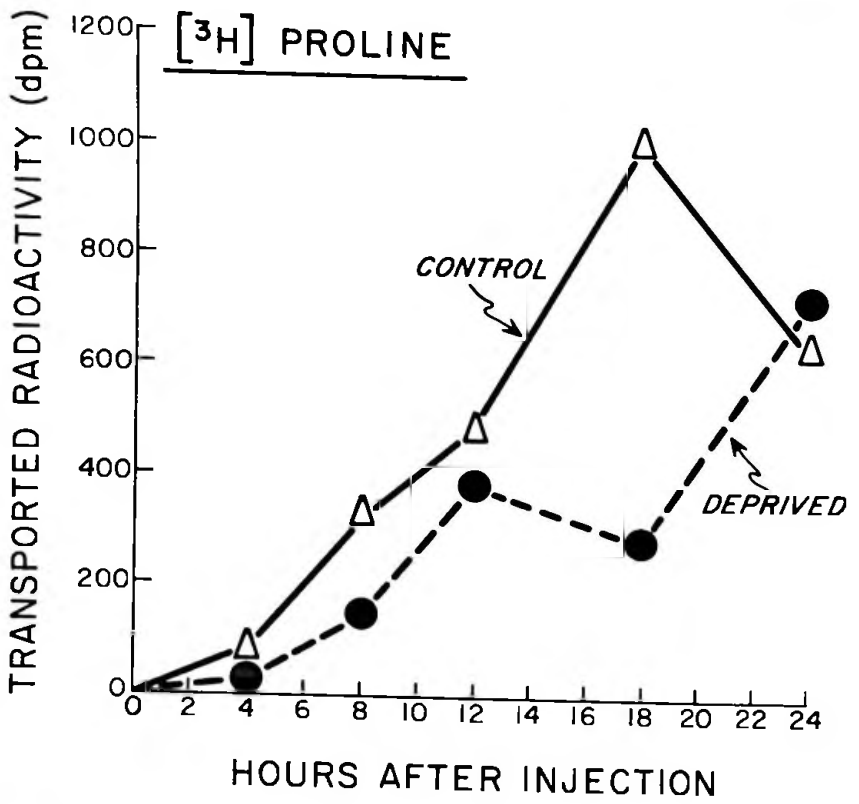

Figure 1. Transported radioactivity in the left tectum at various intervals after injection of (^{3}H) proline into the right eye.

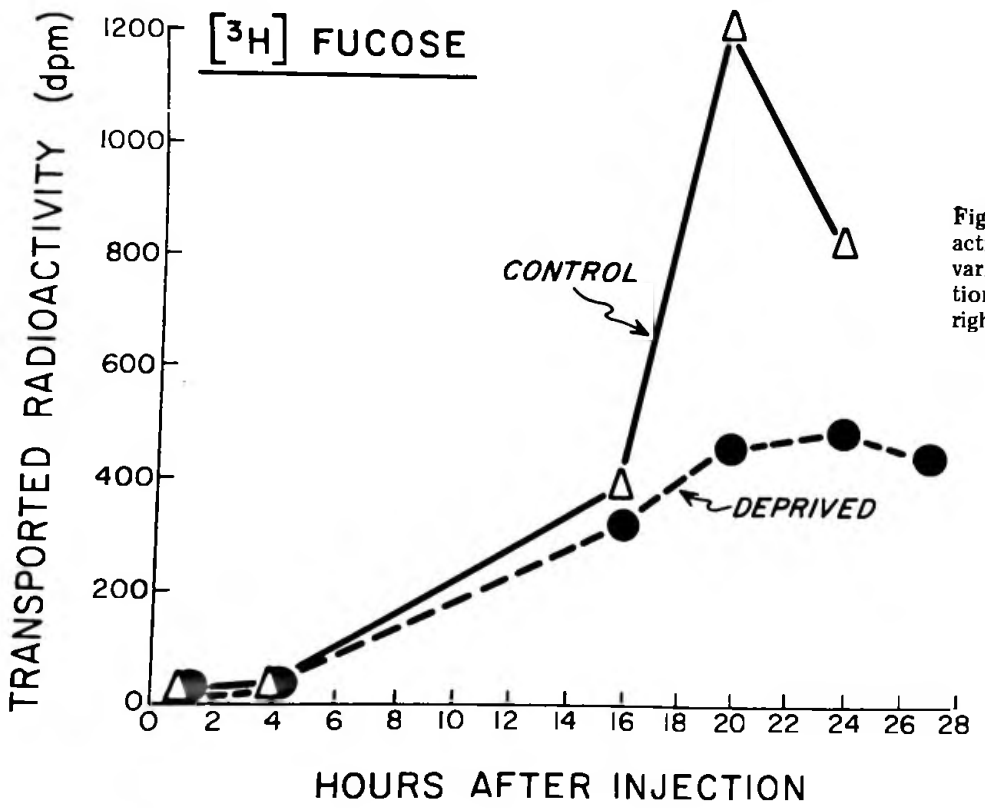

Figure 2. Transported radioactivity in the left tectum at various intervals after injection of (^{3}H) fucose into the right eye.

affect incorporation of amino acids into the ganglion cells (Maraini et al, Expt. Eye Res, 6:299-302, 1967). To check this possiblity proline was injected i.p. into two groups of tadpoles, eye-patched and control. Nine hours later no difference in radioactivity was found between the right and left eyes of the deprived animals or between the right eyes of the deprived animals versus the control animals, suggesting that a lowered incorporation rate does not explain the deprivation effects seen in this study.

These preliminary results suggest that visual deprivation in these developing animals affects the amount of newly-synthesized proteins delivered to the axon terminals of the retinal ganglion cells. Further experiments are required to distinguish between changes in amount and rate of transport. The materials transported with the fast component are largely destined for the synaptic terminals (Droz et al, Brain Research in press 1973) and are constituents of cell membranes (McEwen et al, J. Neurobiology, 2:361, 1971). The lability of fast transport in immature animals may be one portal through which the environment can mold the development of synapses and associated membranous structures. (The author thanks Dr. Marion Murray, of the University of Chicago, for the many helpful suggestions, much encouragement, and the use of her facilities.)

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EFFECT OF RECTAL GLAND EXTIRPATION ON PLASMA SODIUM IN THE SPINY DOGFISH *Squalus Acanthias*

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The chemical composition of the plasma in *Squalus acanthias* appears to be regulated by the interactions of the kidney, rectal gland, and gill. The rectal gland secretes a fluid highly concentrated in sodium with a volume flow of approximately the same magnitude as the urine. Nevertheless it has been suggested that the rectal gland is not essential to homeostasis under resting conditions since in early experiments serum chloride was not consistently elevated after the gland had been extirpated (Burger, J.W., Physiol. Zool. 38, 191, 1965).

The rectal gland of seven dogfish was removed via a small abdominal incision. Seven control animals were sham operated including exteriorization and reinsertion of their rectal glands. All animals were kept in a running sea water tank. Plasma sodium, potassium, and urea were measured daily starting immediately prior to the operation.

The daily postoperative evolution of the plasma sodium is depicted in Figure 1, each dot represents the mean±SE. After removal of the rectal gland there was a rapid and sustained increase in the level of plasma sodium that was significantly different from the control animals. No return towards normal values was observed during the entire experiment lasting eight days. Plasma potassium did not change throughout the experiment nor did plasma urea. All animals eventually died due to reopening of the abdominal incision which was apparent an average of four days after the operation in both experimental and control animals.