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Transport of RNA along the Optic Pathway of the Chick: An Autoradiographic Study

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Summary. Autoradiograms of the optic tectum were made 8 days after monocular injection of ³H-uridine in 3 day-old chicks. Electron microscopic analysis of grain density over morphological structures in various layers of the optic tectum contralateral to the injected eye, was carried out. This revealed that the highest concentration of labeled RNA was within the axons of the more superficial layers of the tectum. A lesser density of grains was observed over glial cells, neuronal perikarya and dendrites. In addition, the bulk of the total grains counted were over axons. In lower tectal layers, radioactivity was predominantly over unmyelinated axons and glial elements. High molecular weight RNA appears to be transported within the axons of the optic nerve toward their terminations which are largely in the outer layer of the optic tract.

Key words: RNA – Axoplasmic transport

Introduction

There is considerable evidence for the proximodistal migration of macromolecular RNA along the nerve fiber (Austin et al., 1966; Rahmann and Wolburg, 1971; Bondy, 1971, 1972; Bondy and Madsen, 1973; Ingolia et al., 1973; Jarlstedt and Karlsson, 1973). However, the migration of RNA precursors and their possible transfer between axons and surrounding glia has made it difficult to definitively demonstrate the intraaxonal migration of RNA (Wolburg, 1972; Autilio-Gambetti et al., 1973; Bondy and Marchisio, 1973). For the resolution of such problems, it is important to know whether the apparently migrating RNA is truly within the axon or not. We have examined the distribution of radioactive RNA in the chick optic tectum following intraocular injection of labeled precursor. This was carried out by autoradiographic means, at a high level of resolution, using electron microscopy. The avian visual pathway has been used because the total crossover of the optic tract leads to each optic lobe being innervated solely from the contralateral eye (Cowan et al., 1961).

This study presents evidence suggesting that the bulk of labeled RNA that appears in the contralateral optic lobe following intraocular injection of radioactive uridine, is within the axons and termini of the primary optic afferents.

Methods

Four three-day old chicks of the White Leghorn strain were injected intraocularly with 10 μ l of an isotonic saline solution containing 250 μ Ci 5- 3 H uridine (New England Nuclear, Inc., Boston, 27 Ci/mM). Chicks were lightly anesthetized with fluothane and injected into the left eye with a 1/2" No. 30 hypodermic needle. Eight days following the injection animals were sacrificed by decapitation. The brains were removed and fixed in 0.1 M cacodylate buffer containing 2% glutaraldehyde. After primary fixation, coronal sections of optic tectal lobe were washed and post-fixed with 1% OsO₄ in veronal acetate buffer containing 7.8% sucrose. The tissue was subsequently dehydrated, infiltrated with Araldite resin and embedded in beam capsules.

For light microscope autoradiography 1 μ sections were cut and mounted on glass slides dubbed with a solution of 0.1% chromic potassium sulfate. Mounted slides were coated with a 1:1 dilution of nuclear emulsion and developed after various exposure times. Tissues were stained with cresyl violet, rinsed in alcohol and dried.

Light microscope autoradiograms were counted for grain density in 5 mm² areas from stratum opticum to the innermost ependymal layer, in a columnar manner. Background was taken as the mean grain density for four 5 mm² areas immediately adjacent to the tissue sample. Four sections were studied from each animal. Net grains per unit area were calculated for ten morphologically identifiable layers. Pretreatment of tissue sections with ribonuclease A (1 mg/ml for 3 hrs at 37° C) before radioautography and staining, effectively removed virtually all the labeled RNA and no silver grains were seen on the autoradiographs. This indicated that the tritium isotope was confined to RNA at the end of the labeling period.

For EM autoradiography the trimmed tissue block was sectioned at 500 Å with a diamond knife on a Porter Blum 11 ultramicrotome. Sections were placed on a 150 mesh nickel grid containing a collodion supporting film. Sections were stained in uranyl acetate and lead citrate then covered with a thin layer of carbon. Ilford L-4 nuclear emulsion was applied with a loop and grids were stored in light-tight boxes at 4° C for 5 months. After exposure the autoradiographs were developed in 13.5% Microdol X, rinsed in distilled water and fixed in 15% sodium thiosulfate for 5 min. The dried autoradiographs were viewed on a Philip's 200 electron microscope.

Light microscope autoradiographs were developed after one, 3 and 5 months to follow the course of brain development and to ensure optimal exposure time for EM autoradiographs. EM autoradiographs from two tectal samples were photographed at 13900 $\times$ by an experienced cell biologist. Random photomicrographs were taken of cross sectional areas of tectal tissue representing 350 μ^2 for each photomicrograph. Grains were counted, in areas representing 17.5 μ^2 tissue area, over dendrites, neuronal cytoplasm and nuclei, myelinated and unmyelinated axons, and in glial cell processes. Grains were assigned localization at the centroid of each grain.

Small 8–10 mg blocks of fixed tissue excised from each tectum of each brain were blotted, weighed and digested in Cellosolve. Tissue digests were counted by liquid scintillation counting in Scintisol at an efficiency of 27% and at 1.5% error, to reaffirm the highly unilateral accumulation of radioactive material in the tectum contralateral to the injected eye.

Results and Discussion

As previously reported (Bondy and Madsen, 1973), the deposition of labeled material in the tectum 8 days following monocular injection of 3 H-uridine was highly unilateral. Tectal tissue ipsilateral to the injected eye had a mean of 542 ($\pm$ 74 S.E.) dpm/mg, whereas the contralateral tectum had a mean of 6542

Fig. 1. Autoradiographic grain density of layers of the right chick optic tectum. Sections were made 8 days after injection of 250 μCi (^3H)-uridine into the left eye, and these were exposed to emulsion for 30 days. Columns represent the radioactivity of various layers and are the mean density (per unit area) of sections of each of 2 optic tecta. Morphological layers as follows: 1, ependyma; 3, stratum fibrosum periventriculare; 4, molecular zone; 5, stratum griseum periventriculare; 6, 7, stratum album centrale; 8, stratum griseum centrale; 9, 10, molecular zone; 11-12, stratum fibrosum griseum; 13-14, stratum opticum

(± 1477 S.E.) dpm/mg for the four brains. Light autoradiography revealed that the majority of silver grains were localized in the outer layers of the tectum with declining density with direction away from the stratum opticum (Fig. 1).

Electron micrographs were taken randomly in representative areas of stratum opticum (Fig. 2a) and in the four morphological layers underlying the stratum opticum (Fig. 2b). In the stratum opticum the majority of grains appeared over neuronal axons (Fig. 3). Only 16% of the grains appeared over glial processes whereas 76% were localized within the axons. In the ventral area of the stratum opticum some neuronal cell bodies and dendrites showed grain accumulation as well.

The deeper tectal layers showed accumulation of grains over axons, and a roughly equal accumulation over glia (Fig. 2b). The accumulation of grains

Fig. 2. Electron microscopic analysis of grain density of layers of the right chick optic tectum. Sections made as described in Figure 1, were exposed to emulsion for 160 days. Grains were counted in **a** 8 plates of the stratum optic region and **b** 16 plates of regions 2–5 of each of two samples of tecta at a magnification of 13 800. Columns represent grain densities over various morphological structures. Bars indicate standard error of the mean

over neuronal soma and dendrites was low. It is unlikely that grains over axons simply represented “scatter” from surrounding glial processes. The source of glial labeling may represent local *de novo* synthesis from a soluble precursor. The axoplasmic transport of nucleotides has been previously shown (Bondy and Marchisio, 1973).

The presence of a population of labeled unmyelinated axons in the lower tectal layers suggests that fibers of the optic pathway may terminate deeper than has been detected by degeneration studies. This is also suggested by an earlier light microscopic study (Bondy and Madsen, 1973). Autoradiography may be an especially sensitive means of tracing neuronal pathways and may reveal tracts not traceable by degeneration studies (Cowan et al., 1972). However, we have not definitively established the origin of these deeper, labeled axons which are seen down to the stratum griseum centrale.

Autilio-Gambetti et al. (1973) injected rabbits intraorbitally with labeled uridine and then carried out autoradiography of the optic nerve. After 9–17 days the heaviest labeling was over glia and 80% of grains representing RNA were outside axons. These studies suggested that a large proportion of intra-

Fig. 2b

ocularly injected uridine was incorporated into RNA synthesized in the optic tract by glial cells adjacent to axons. The remaining labeled RNA was present intraaxonally and its site of synthesis was unclear. The migrating rate reported by these workers was much more rapid (31 mm/day) than the rate (10–15 mm/day) we have obtained (Bondy and Madsen, 1973). Differences between our data and that of Autilio-Gambetti's group may also be due in part to the much greater emphasis given the primary visual pathway in birds in comparison to the rabbit.

Our data indicate that the accumulation of grains over axons represents 89% of the total grains in the stratum opticum and 65% of the total grains over axons in deeper tectal layers. Only 34% of grains appearing over neuronal structures were over postsynaptic structures while 65% were over presynaptic structures (axons as opposed to dendrites and nerve cell bodies).

Autoradiographic methods cannot show with precision, the chemical nature of the substances causing emulsion decomposition. However, we have previously examined the biochemical characteristics of the radioactive material found in the chick optic tectum after intraocular injection of ^3H -uridine. This appears to largely consist of ribosomal RNA. Since ribosomal protein also seems to migrate in a similar manner, it may be that ribosomes move distally along the axon (Bondy and Purdy 1975). The transport of ribosomal RNA is further substantiated by the inhibition of such apparent transport following intraocular injection of a very low dose of actinomycin D, which selectively

Fig. 3. Electron microscopic autoradiography of stratum opticum of right chick optic tectum. Sections were made as described in the text. Magnification = 13 800

blocks ribosomal RNA synthesis (Bondy and Purdy, 1975). Furthermore, the stability of apparently translocated RNA in the optic tectum, depends on the integrity of the optic nerve (Bondy and Purdy, 1975). This also suggests the presynaptic location of such RNA.

The biochemical data, taken together with the radioautographic results presented here strongly imply that RNA synthesized in retinal ganglion cell bodies may migrate within the axons of the optic pathway and thus appear in the innervated optic lobe.

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