LASER-DEGENERATION STUDY OF NERVE FIBERS IN THE OPTIC NERVE.

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LASER - DEGENERATION STUDY OF NERVE FIBERS IN THE OPTIC NERVE

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1. Introduction

Knowledge about wiring of neurons is one of the most important goals of neurobiology. Neuronal processes -axons and dendrites- degenerate when they are severed from their cell body. Since different staining procedures distinguish between the degenerating axons and their healthy neighbors, most neuroanatomical pathways have been mapped through the follow-up of degenerating axons after spontaneous or experimental lesions at some point of the pathway. Mapping of neuroanatomical connections has been enormously enriched during the past few years, thanks to new labelling techniques with great resolution power [1]. However, the resolution of the older degeneration procedures is only limited by the extent of the lesion and the resolution of the differential staining of degenerating axons. As we will show in this report, the use of a laser to produce small lesions in the retina of birds, coupled to the detection of degenerating axons in semi-thin plastic sections [2] is allowing us to understand the relationship between axons along the optic pathway with a resolution comparable to that of "in vivo" labelling techniques.

Wiring between the retina and the brain is established during early embryonic life, when a population of neurons -the ganglion cells- develops

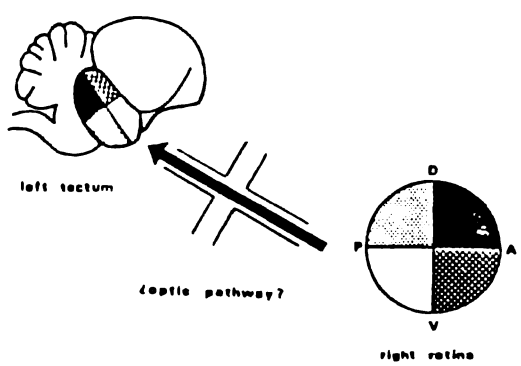

Figure 1. Each point of the retina project to a corresponding point of the optic tectum. It is not known whether the optic axons maintain a retinotopic order along the optic pathways or whether they are organized according to other rules.

in the retina. Each ganglion cell has an axon which grows to the dorsal wall of the mesencephalon -the optic tectum- where it finds an appropriate target neuron. These connections are not formed at random, since each point of the retina is matched to a precise point of the optic tectum (Figure 1). However, we still do not know those factors controlling the organization of these connections. One of the possible explanations is that axons leave the retina in an ordered fashion and that they keep the same neighbors along the optic pathway [3,4]. If this were the case, one should find an ordered distribution of optic axons along the visual pathways. Accordingly, after the lesion of one (or a portion of) of the quadrants of the retina shown in Figure 1, one should find a similar distribution of degenerating axons in the optic nerve. Our studies of the optic nerve after laser lesions of the retina indicated that this is not so in the visual pathways of quails.

2. Material and Methods

Four week-old quails (Coturnix coturnix) were anesthesized by an intraperitoneal injection of chloral hydrate (25 mg/100 g. body weight) and procaine (30 mg/100 g. body weight). The right eye was opened with a lid retractor after local anesthesia of the cornea with tetracaine. The beam of a Spectra Physics Ion Laser, model 165, operated at 514,5 nm, was focused to a 100 μm point on the surface of the cornea by a lens system. Various light energies and exposure times were used, and conditions for each experiment are detailed under the corresponding figures. Quails were killed by decapitation one week after irradiation. The position and size of the lesion was determined in flat-mounted retinas which were stained with cresyl violet. Optic nerves were fixed in 2.5% glutaraldehyde in 0.09 M cacodylate buffer with 0.12 M sucrose for 24 hours. After dehydration, nerve slices were embedded in epoxy resins. Consecutive 1.5 μm sections were serially mounted and stained with 1% p-phenylendiamine [2] for light microscopic studies. Ultrathin sections for electron microscopy were stained with lead salts.

3. Results and Conclusions

A week after irradiation, lesions appeared as holes perforating all layers of the retina. Figures 2 and 3 show one of these lesions placed to the nasal side of the pecten, which is the vascular structure lying over the nerve papilla. The retina of birds has no intrinsic vessels and retinal damage was restricted to the beam absorption area. Also affected were those ganglion cells from more peripheral regions whose axons passed through the damaged area in their route to the nerve papilla. Since most axons take a more or less straight pathway to the nerve papilla, a circular sector of the retina was disconnected from brain. The size of this sector depended on exposure times, laser energy output and the distance between the absorption area and the nerve papilla. Obviously, the more peripheric lesions disconnected fewer ganglion cells than those placed on a central position closer to the retina. Thus, laser irradiation made it possible to eliminate as many ganglion cells as it was desired without any alteration of ocular geometry such as would occur after conventional surgical procedures. By the same token, hemorrhagic and infectious complications were minimal.

The optic nerve had an elliptical shape and was surrounded by a thick

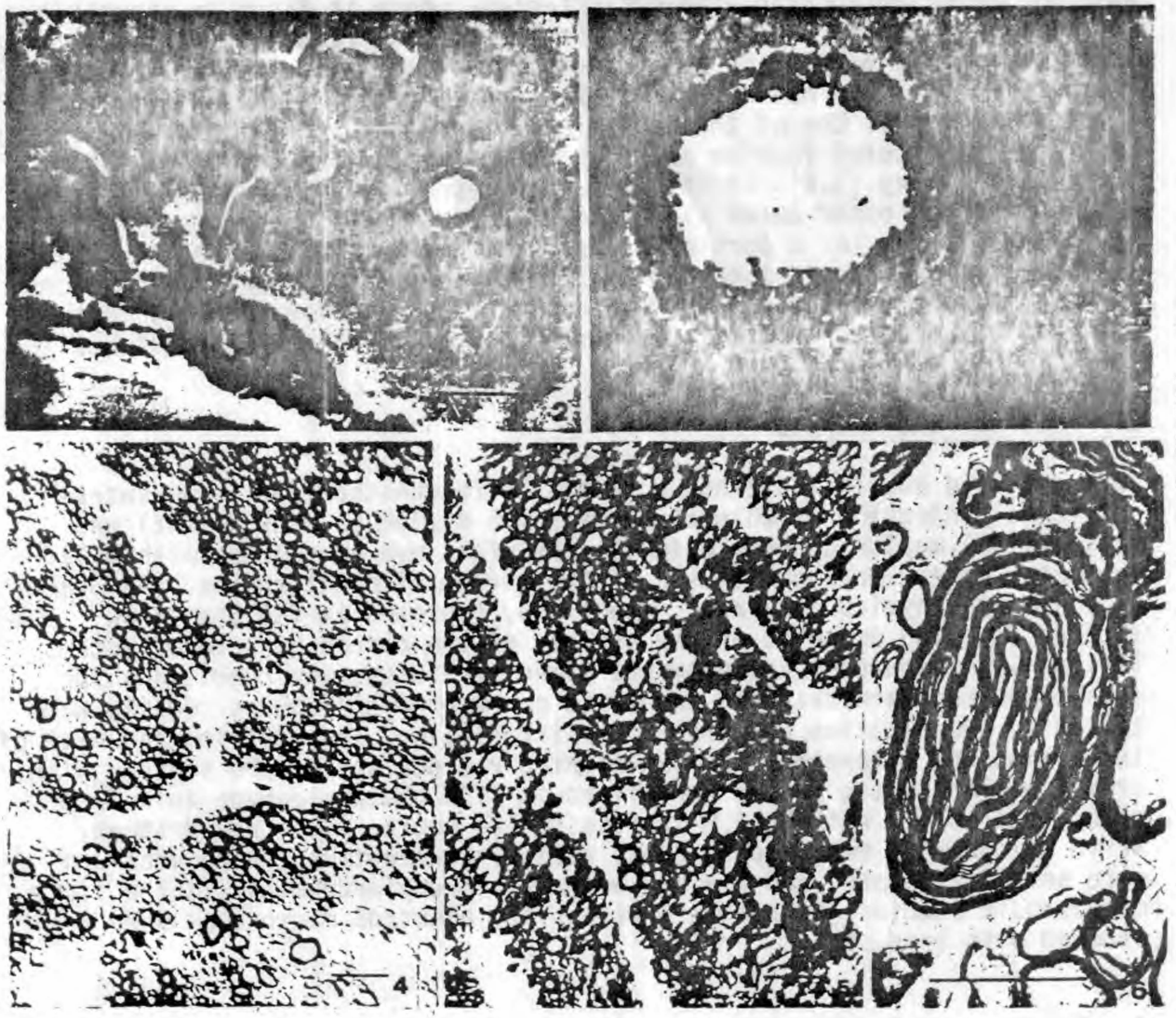

Figure 2 and 3. A lesion in the ventronasal region of the retina made by an irradiation of 0.5 watts for one second. The lesion appeared as a hole surrounded by reactive pigmented cells. The pecten is the dark structure at the botton of Figure 2. The striated pattern of the retinal surface reflects the pathway of optic axons which leave the eye through the nerve papilla. The latter lies beneath the pecten. Calibration bars 1 mm and 0.2 mm.

Figure 4. Light micrograph of a normal nerve showing myelinated axons of different diameters. Calibration bar 10 µm

Figure 5. A nerve showing degenerating fibers. The arrows indicate axons with degeneration of the clear type. Dark spots correspond to dark degenerating axons. Calibration bar 10 µm.

Figure 6. An electron micrograph showing the disappearance of axoplasm and the disruption of myelin sheath in a degenerating axon. Calibration bar 1 µm.

connective sheath. Connective septa were also found within the nerve but they were placed at random. The nerve contained both myelinated and unmyelinated axons of different sizes (Figure 4). The presence of degenerating fibers could be recognized even in unstained preparations, since many of these dying axons had larger profiles than their normal counterparts. These clear degenerating axons were interspersed with dark degenerating axons -i.e.: those binding more osmium tretoxide- which were clearly recognized by their deep brown colour in p-phenylendiamine stained preparations (Figure 5). Preliminar studies showed that one week survival time gave an optimal picture of degeneration, since axonal fragmentation was almost absent before that period. These findings were confirmed by transmission electron microscopy which showed that both the enlarged profiles and the dark spots of light microscopy belonged to degenerating axons (Figure 6).

Sections of optic nerves were examined with a 1,000 X magnification and the number of dark degenerating fibers per unit area was determined at points separated by fixed intervals of the microscope stage. In this fashion, density maps like those shown in Figure 7 were obtained. The distribution of degenerating fibers varied according to the localization of the lesion on the retinal surface. In some cases (Figure 7 A), the degenerating fibers remained as a group. However, they did not resemble the sectorial shape of the retinal lesion but appeared as stripes across the anteroposterior aspects of the nerve, suggesting that some change in the distribution of the axons had accurred along the pathway. These strip distributions were associated with lesions in the ventronasal region of the retina. When lesions were made in a more dorsal portion of the retina,

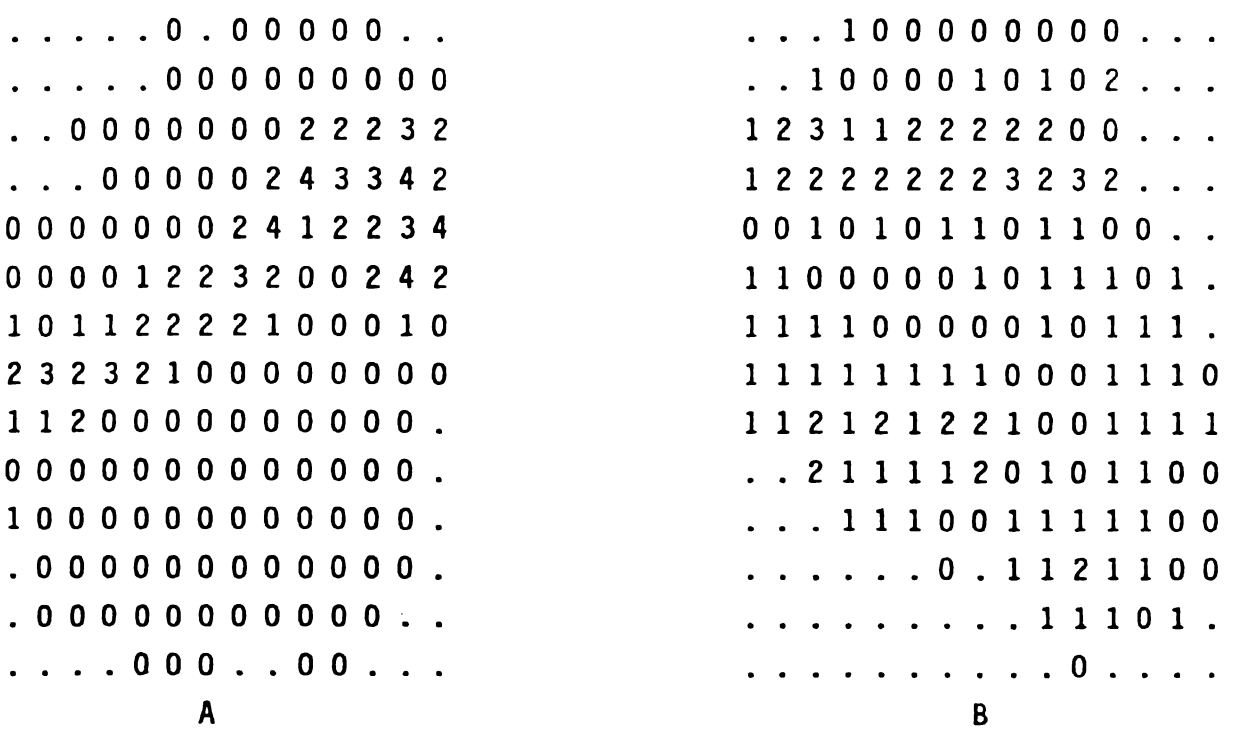

Figure 7. Densities of degenerating fibers one week after irradiation with 1.8 watts for 10 seconds. The numbers indicate the amount of dark degenerating terminals on an arbitrarily defined area (0 = background; 1 = 9-20; 2 = 21-32; 3 = 33-44; 4 = 45 or more). (A) Strip patterns found after lesions in the ventronasal region; (B) diffuse patterns found after lesions in the dorsal region of the retina.

degenerating fibers were spread abroad most of the cross section of the optic nerve (Figure 7 B), even though the lesions never affected more than 1/5 of the ganglion cell population. The existence of these diffuse patterns was a clear evidence that at least some of the axons lost contact with their neighbors.

Our observations indicated that there is not a strict retinotopic order in the optic nerve of quails. A lack of such organization has also been observed in cats [5] and it has recently been concluded that the order found in the optic nerve of goldfish is of a chronological nature [6]. Further studies are neccessary to ascertain the biological significance of strip and diffuse degenerating patterns in the optic nerve of quails. However, it can be speculated that they represent two different mechanisms. The guidance of at least part of the fibers would not depend on the maintenance of neighbourhood relationship during the migration of axons.

On the other hand, it should be emphasized that the resolution of this procedure can be further increased, since it is possible to make smaller lesions than the ones reported here. Particularly promising is the study of very small lesions which would not affect axons passing across the absorption area of the laser beam.

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