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Neurofibrillar Changes in Goldfish (Carassius auratus L.) Brain in Relation to Environmental Temperature

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Summary. Neurofibrillar changes occur in the brains of goldfish, Carassius auratus L., maintained at 5° C for 177 days or more under a 12-hour photoperiod. In paraffin sections impregnated by the silver method of Holmes, the light microscopic appearance of the neurofibrillary change was evidenced by black rings 1 μm to 2 μm in outside diameter. A quantitative study showed the mean number of rings to be: optic tectum layer 3, $1.34 \times 10^2/mm^3$, layer 5, $1.14 \times 10^2/mm^3$, nucleus prerotundus $4.63 \times 10^2/mm^3$, olfactory bulbs, $6.2 \times 10^2/mm^3$. In the brains of fish kept at 15° C a few rings are found only in some olfactory bulbs and not in any other region of the brain. The brain wet weight also changes and was found to be significantly ($p < 0.01$) less in 5° C than in 15° C fish, the mean values being 0.1588 g and 0.2091 g respectively.

The significance of the observed changes is discussed. It is suggested that the smaller brain wet weight may reflect a change in the vasculature of the brain and be related to the acclimation process. It is hypothesized that the rings are a morphological expression of a physiological change caused by prolonged exposure to low temperatures; a neuronal response reflecting either early degeneration or a functional adaptation which may involve an alteration in axoplasmic transport. It is suggested that this system is a useful model for the study of relationships between neurofibrillar disturbances and neuronal function.

Key words: Neurofibrillar rings – Temperature – Goldfish

Introduction

The effect of environmental alteration on neurofibrillar structures in the brains of reptiles has been studied by Tello (1904), Cajal (1904), Boycott and Guillery (1959), Boycott, Gray and Guillery (1961), and more recently by Potter (1973) and Potter and Hafner (1974).

In some poikilotherms, namely the lizards, Lacerta viridis (Boycott and Guillery, 1959; Boycott et al., 1961), and Sceloporus undulatus (Potter, 1973),

the bullfrog, R. catesbeiana (Potter and Hafner, 1974) and the green sunfish, Lepomis cyanellus, (Roots, unpublished observation), the number of ring-like neurofibrillar structures in the brain is quantitatively related to environmental temperature, the number of rings being greater at lower temperatures. The magnitude of the change ranges from a two-to-three-fold increase in the number of rings in the hippocampus to a thirty-fold increase in the molecular layer of the cerebellum of lizards.

Goldfish were chosen for the present quantitative study because of their tolerance of a wide range of environmental temperature. Furthermore, with the exception of the olfactory bulbs neurofibrillar rings are not present in brains of goldfish maintained at an ambient temperature of 15° C. Thus any ring appearing on exposure of the fish to low temperatures can be regarded as a physiological response to the lowered temperature.

Methods

Goldfish, Carassius auratus L., 90 mm to 120 mm in body length were obtained from Canadian Goldfish Gardens and Supply, Toronto, Ontario. Six fish were placed in each of five 15-gallon tanks containing filtered dechlorinated aerated water, in a constant temperature environmental room, at 15° C ± 1° C on a 12-hour photoperiod for 28 days. The fish were fed with Purina Trout Chow no. 2 daily.

The fish were then divided into two groups as follows: 1. 10 fish (NT fish) were placed five each in two 15-gallon tanks, and maintained in a constant temperature environmental room at 15° C ± 1° C on a 12-hour photoperiod; 2. 20 fish (CT fish) were kept at 5° C ± 1° C. Fish were fed daily at 15° C and every other day at 5° C with Purina Trout Chow no. 2. The tank water was changed at intervals of 21 days. The goldfish were exposed to the appropriate temperatures for 177–236 days.

The fish were weighed. The brain and olfactory bulbs of the 30 goldfish were dissected out of the cranial cavity within sixty seconds of decapitation, and immediately immersed in pre-weighed vials of the fixative, 2.5% glutaraldehyde in Karlsson and Schultz phosphate buffer (Karlsson and Schultz, 1965; Schultz and Karlsson, 1965). The vials were then reweighed to obtain the wet weight of the brains.

After 12 hours, the fixative was replaced by fresh 2.5% glutaraldehyde in buffer in which the tissue remained for 24 hours before further processing. Each brain was processed through tetrahydrofuran (THF) (Haust, 1958) to paraffin wax. Holmes' silver staining procedure (Holmes, 1943) was used as Downes (1971) reported that the fixation of brain tissues with glutaraldehyde gives unreproducible results when used in combination with ammoniacal silver techniques. A modification of Holmes' silver staining method when applied to fish tissue was necessary. The boric acid-borax buffer was adjusted to pH 8.3, in contrast to the pH of 8.4–8.5 for formalin-fixed human nervous tissue. A reduced toning time in gold chloride was used, followed by a decrease in concentration of the oxalic acid reducer from 5% to 1%.

Serial sagittal sections of brain tissue of experimental fish for silver impregnation were cut at a microtome setting of 10 μm. These sections were used to obtain quantitative values for the ring-like structures appearing in the goldfish brain. The nucleus prerotundus is approximately 250 μm wide in processed brain tissue. Therefore, twenty-five 10 μm thick serial sagittal sections through the nucleus prerotundus in each fish were selected for ring counts. As the optic tectum is curved, it is not cut perpendicular to the layers in every sagittal section. In the 25 serial sagittal sections that were used for counts in the nucleus prerotundus, ring counts were made for the optic tectum. The plane of sectioning is perpendicular to the layers of the optic tectum in these sections. The olfactory bulbs were serially sectioned transversely.

The sections were examined with a Reichert Zetopan binocular microscope with a micrometer disc in the right eyepiece. Rings were counted under a 40× objective lens and 10× eyepiece. The area of the region containing the rings was obtained with a grid in place of the micrometer disc in the

right eyepiece. A green filter was used for all ring counts; the resolution of the 40× objective lens was determined by the formula:

$$h = \frac{0.61\lambda}{NA}$$

where h represents the minimum resolvable distance (0.5 μ):

λ represents the wavelength 5000–5500Å (for the green filter) of the light illuminating the slide;

and NA represents the numerical aperture (0.65 for the 40× objective lens used) (Martin and Welford, 1971; Willey, 1971).

Ring counts are expressed as number per slice volume (cubic millimeter) according to the formula:

$$n_v = \frac{N}{T.A}$$

where n_v represents the number per slice volume;

N represents the number of rings counted;

T represents the thickness of the section;

A represents the area scanned for rings (Weibel, 1967).

Neuronatomical features of the brain were determined from the silver-stained serial sections and from sections of two other brains, one of which was sectioned transversely and the other sagitally at 5 μm. Alternate groups of five serial sections from each brain were stained with luxol fast blue-periodic acid Schiff (after Klüver and Barrera, 1953) or with haematoxylin and eosin. These sections were compared with the descriptions and definitions of areas made by Sheldon (1912) and Tuge, Uchihashi and Shimamura (1968).

Results

The mean body weight of the NT fish was 53.59 g and that of the CT fish was 60.62 g. The difference is not significant. However, the difference between the mean brain wet weights, 0.2091 g for NT fish and 0.1588 g for CT fish is significant ($P < 0.01$).

In the olfactory bulbs, rings appear in both NT fish and CT fish (Figs. 1 and 2). Only one or two thin-walled rings are visible in three of the NT fish sections whereas six to twelve rings of small, or large, thick- and thin-walled types are present in all CT fish sections. The mean number of rings in CT fish is $62/mm^3$. In both CT and NT fish the rings are distributed throughout the sections and do not appear to be confined to any specific neuroanatomical area.

In the brain exclusive of olfactory bulbs, rings are present only in fish kept at 5° C for 177 days or more. The areas of the CT fish brain in which the ring images appear most consistently are: optic tecta, pars glandularis of the hypophysis, nucleus rotundus complex and corpora mammilare.

The nucleus prerotundus and immediately adjacent tissue contain the most rings per volume of tissue (Fig. 3). The mean number of rings is $4.63 \times 10^2/mm^3$. Counts of rings in serial sections reveal that the number of rings was not uniform throughout the nucleus prerotundus. The majority of the rings in this nucleus is located ventral to the optic tract in an area devoid of cell nuclei and with few capillaries and myelinated fibers. There is a variability in thickness of the walls of the rings, in that large or small rings may display either thick or thin black outlines. In all rings, the central area appears to be free of silver-impregnated material; the outside diameter of the rings ranges from 1 μm

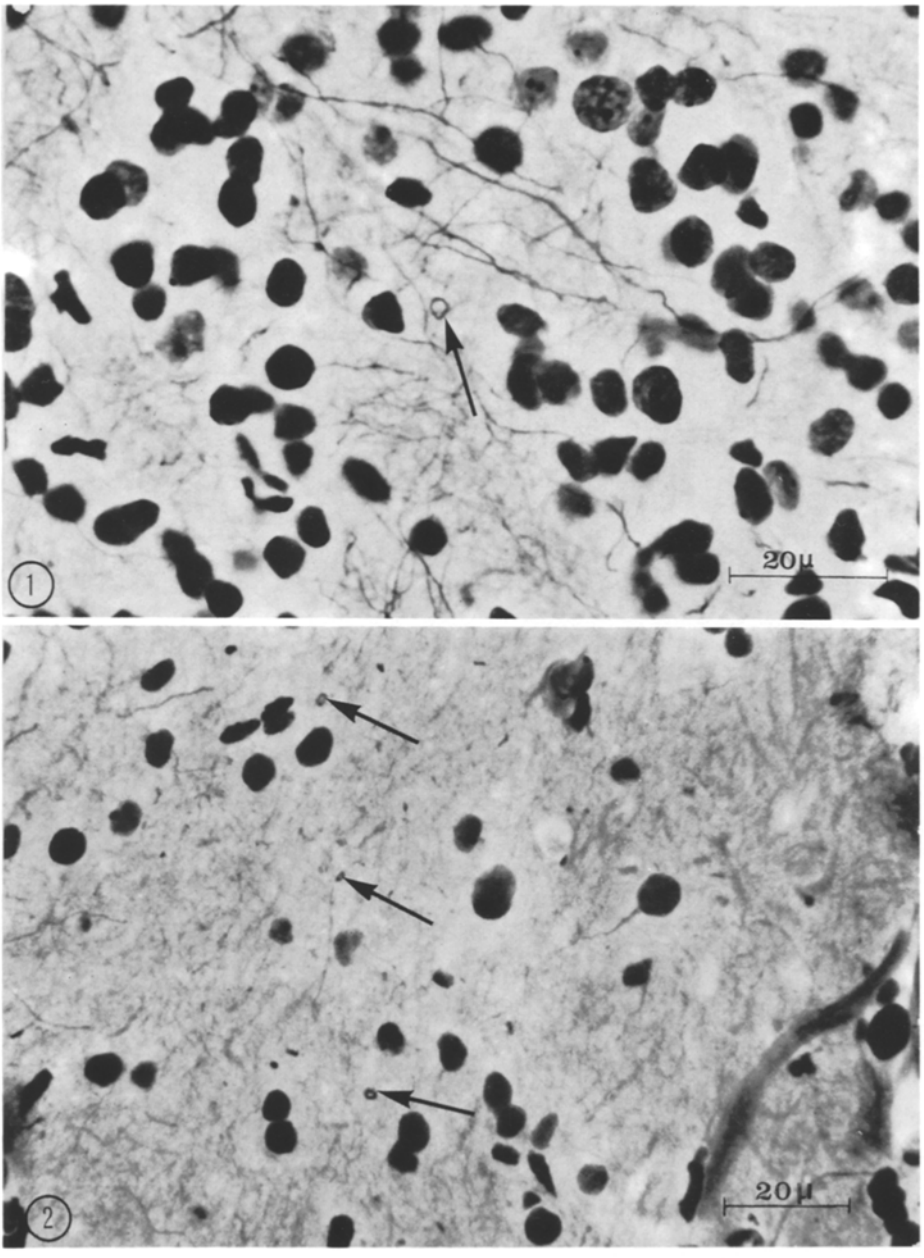

Fig. 1. Section of olfactory bulb from NT fish with a thin-walled ring (arrow)

Fig. 2. Section of olfactory bulb from CT fish with many rings (arrows)

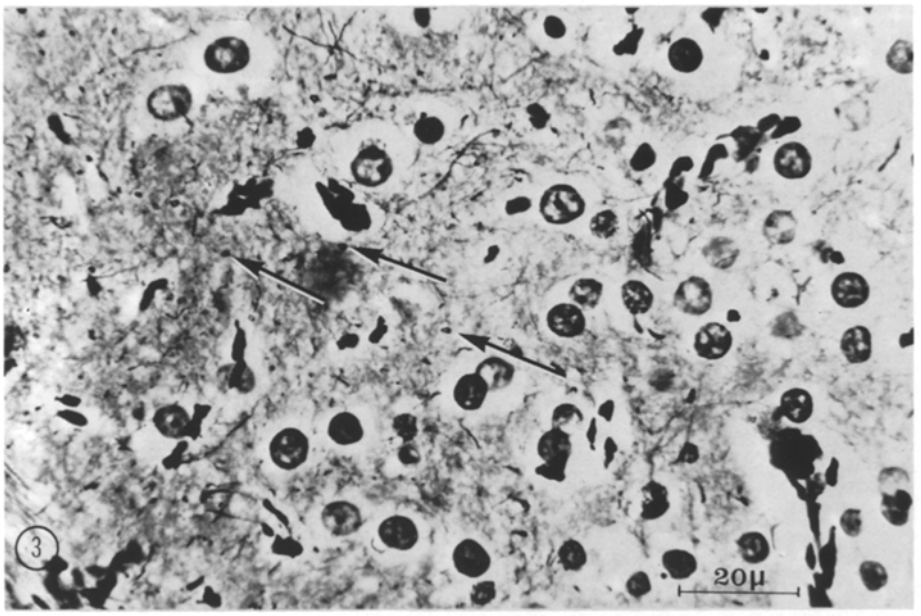

Fig. 3. Photomicrograph of the cells and rings (arrows) in the nucleus prerotundus complex of a CT fish

to 3 μm. Rings appeared at all levels of focus indicating that they are not optical sections of spheres.

Occasional ring structures are observed in the mammillary bodies in CT fish, but not in NT fish. Throughout the rest of the lateral lobes only a few ring structures can be identified.

In the optic tectum ring structures are located in layer five (Fig. 4) and layer three (Fig. 5). In layer five the rings are sparse and range from 1 μm to 3 μm in outside diameter. Although most of the rings have thick darkly staining walls, and a clear centre, a few rings have thinner walls (Fig. 6). The mean number of rings in this layer is $1.14 \times 10^2/mm^3$. The rings in layer three are more numerous than in layer five, the mean number being $1.34 \times 10^2/mm^3$. In layer five the rings are distributed throughout, whereas in layer three, the majority of the rings are dispersed throughout the posterior region with a few scattered rings in the anterior region. The rings in layer three are of the same dimensions and morphological appearance as those in layer five.

Discussion

Boycott et al. (1959, 1961) reported the occurrence of neurofibrillar rings in the hippocampus, cochlear grey, and the superior olivary nucleus. The hippocampal rings, 1 μm to 2 μm in diameter lie on the cell bodies of the large-cell layer or near to basal parts of the apical dendrites. The rings in the cochlear grey are larger (often 4 μm in diameter), more irregular in shape, and are seldom related

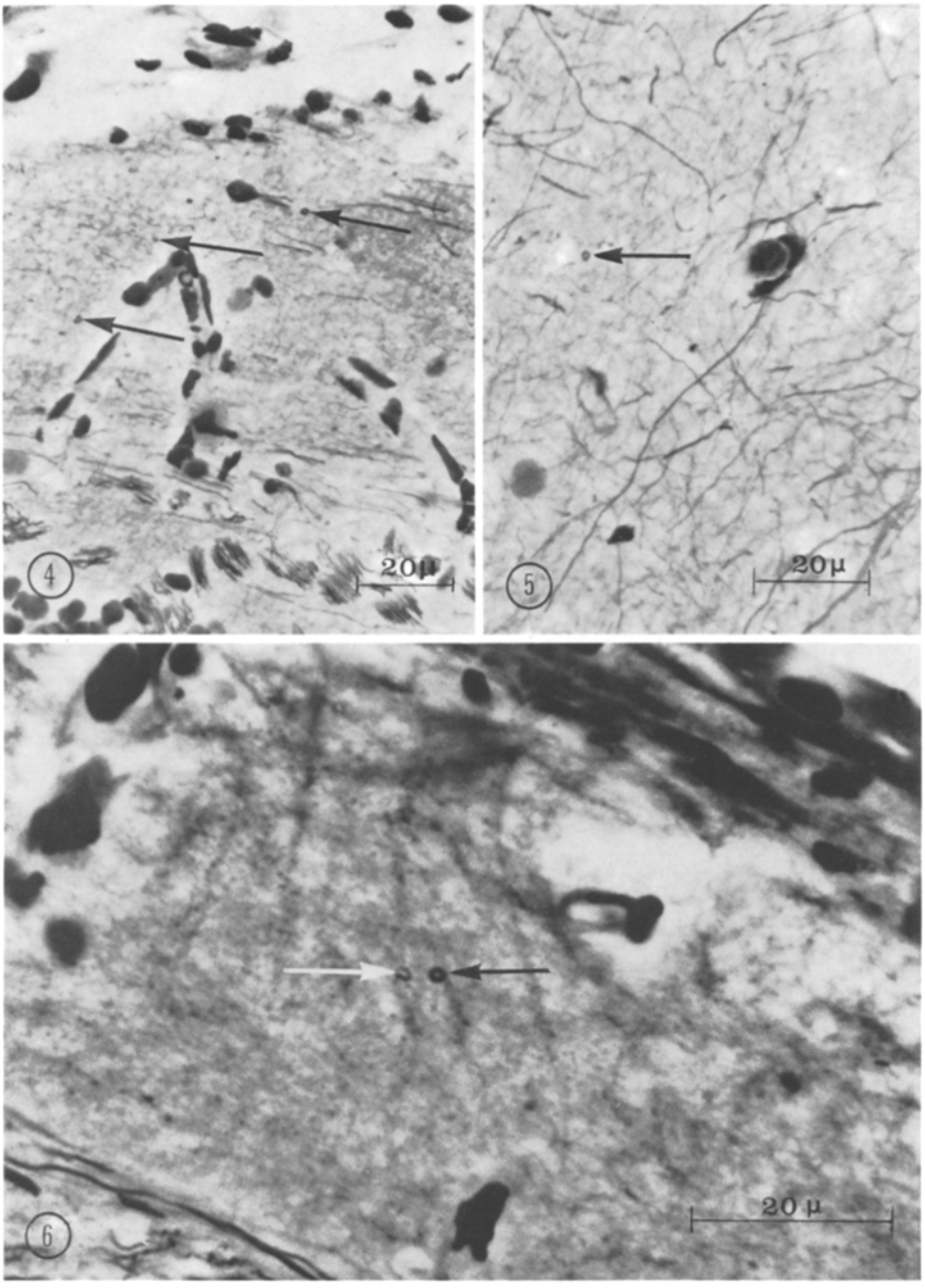

Fig. 4. Section of optic tectum of CT fish showing the large number of rings in the posterior portion of layer five

Fig. 5. Section of optic tectum of CT fish showing a thick-walled ring (arrow) in layer three

Fig. 6. Section of optic tectum of CT fish showing a thick-walled ring (black arrow) and a thin-walled ring (white arrow) in layer five

to perikarya. Most rings in the superior olive are smaller and more closely packed than those in the cochlear grey, while other rings are more similar to the cochlear rings in size.

The ring counts were made on sections of brains of lizards maintained at 19° C and 32° C, and of lizards rewarmed to 32° C after exposure to 19° C for eight weeks or more. In the cochlear grey, the superior olivary nucleus and the hippocampus there was a loss of neurofibrillar rings in brains of lizards kept at high temperatures. Boycott et al. (1961) noted however that the neurofibrillar rings in the cold lizards take up the silver differently than the rings in the warm lizards.

In most major synaptic regions in sections from cold acclimated lizards, counts of 60 rings/$10^4 \mu m^3$ ($6 \times 10^6/mm^3$) were obtained, whereas 20 rings/10^4 μm^3 (2×10^6 mm^3) were present in warm acclimated lizards. It is important to note that in the present study on goldfish brains, ring structures were observed only in some olfactory bulbs of 15° C fish and not in any other region of the brain. After 177 days exposure to 5° C, goldfish brains exhibited most neurofibrillar ring structures in the olfactory bulbs, nucleus prerotundus, and layers three and five of the optic tectum.

The ring counts determined for goldfish brains in the present study are 10^3 to 10^4 smaller than counts reported by either Boycott et al. (1961) for Lacerta, or Potter (1973) for Sceloporus.

Although present techniques do not permit definative correlation of neurofibrils with neurofilaments it is highly probable that the rings represent accumulations of neurofilaments (Boycott et al., 1961; Roots and Bondar, 1977). The appearance of two types of ring structure, a thin-walled type and a thick-walled variety, may be explained on the basis of quantity of argyrophilic material present in each ring. In the darker staining walls, more neurofibrillar material is available than in the rings with thin black walls. It is also possible that the thin structures are really areas where an axon becomes tortuous. According to Pecci-Saavedra, Vaccarezza and Mascitti (1969) and Walberg (1971), ring structures may also be formed by silver precipitates located in anoxic axoplasm which may have undergone some physiochemical changes during degeneration.

Perikaryal neurofibrillary degeneration has been reported in anoxic tissue cultures of chick spinal cord neurons (Kim, 1971). The degree of neurofibrillary alteration increases with length of exposure to anoxia. Chronic hypoxia in rats produces tubular changes in the axoplasm of many nerve fibers, and enlarged produces tubular changes in the axoplasm of many nerve fibers, and enlarged presynaptic terminals containing either multilamellar bodies or clumped vesicles be reflected in changes in the neurotubules or neurofilaments in the neuron and its processes.

It appears reasonable that any change in the oxygen level of an area of the brain will elicit some form of neuronal response to the altered environment. In ground squirrels during hibernation, there is a reduction in the capillary supply (Drummond, 1962) in response to and in proportion to reduced functional requirements. Ring structures noted in Bodian stained sections of formal-dehyde-fixed ground squirrel hibernator brains are not present in the brains of active squirrels (Mrosovsky and Roots, 1977). The correlation of the

appearance of ring-structures and reduction in the capillary supply during hibernation has not been evaluated. The presence of neurofibrillar thickening in hibernating reptiles is reported by Tello (1904) and Cajal (1904), and in cold acclimated lizards by Boycott and Guillery (1959) and by Potter (1973). However, ring structures are also present in brains of lizards maintained at normal environmental temperatures.

A change in the vasculature of the brain, in terms of vasoconstriction, might be a possible explanation for a neuronal response to decreased oxygen levels, in the form of neurofilaments appearing in the terminals. The decrease in brain weights of CT fish with respect to NT fish may reflect a change in blood volume. The vasoconstriction could be highly reversible, with a rapid increase in blood volume upon exposure of the fish to warmer temperatures. Regional differences in the rate of appearance and disappearance of the rings in lizards (Boycott et al., 1961) and bullfrogs (Potter and Hafner, 1974) may be due to differences in the microcirculation .

The area containing the most rings, nucleus prerotundus, is thought to be related to feeding. A decrease in the activity of the CT fish would result in a subsequent decrease in function of nuclear areas involved in the process of feeding. A somewhat similar explanation may be put forth to account for the appearance of rings in other areas of the brain, i.e., a slow-down of neurological activity through lessened requirements. The rings, then, may be in areas associated with long-term adaptation to the cold.

Neurofibrillar material accumulates in some pathological conditions. Until now it has been impossible to study the effect of this accumulation on neuronal function since the methods available to induce changes in neurofibrillar material (injection of mitotic spindle inhibitors and alkaloids) block normal functions of other dependent systems. Since neurofibrillar rings are not present in the brains of goldfish kept at moderate and high temperatures, and appear only after prolonged exposure to low temperatures, it is possible to separate changes occurring as the result of the appearance of neurofibrillar rings from temperature-dependent physiological activity. The system thus provides a useful model for the study of relationships between neurofibrillar changes and neuronal function. Effects on axoplasmic transport are currently being investigated.

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