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The development of the optic tectum in *Xenopus laevis*: a Golgi study

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INTRODUCTION

In a study of the effect of eye removal in frogs on the development of the optic tectum, Kollros (1953) reported that the highest number of mitotic figures was to be found in the caudomedial part of the tectum during normal development. More recently Straznicky & Gaze (1972), using an autoradiographic technique, have confirmed this general conclusion in *Xenopus laevis*, and have discussed the significance of this phenomenon in the establishment of specific neuronal connexions. Further studies (Gaze, Chung & Keating, 1972) have revealed that from stage 44 (Nieuwkoop & Faber, 1965) of larval life onwards the organization of the retinotectal projection changes, resulting in a shift of the projection of the central visual field caudalwards, and a reduction in the size of multiunit receptive fields. Using a degeneration technique, Scott (1973) has found complete tectal coverage by ingrowing optic fibres only after stage 60. Chung, Gaze & Stirling (1973) have reported changes in the characteristic features of single tectal units during the development of young tadpoles through metamorphosis.

These physiological studies demand a deeper insight into the structure of the developing tectum. The present study is an attempt to collect information about structural changes at the neuronal level in the optic tectum during larval life.

MATERIALS AND METHODS

The rapid Golgi technique was used to stain nerve cells in the brains of the toad *Xenopus laevis*. In younger tadpoles (between stages 46 and 55) the staining procedure was usually repeated twice or three times, according to Valverde (1965). Later than stage 55 and in adults the single treatment was applied. In these cases the fixation time in the mixture of 2% potassium dichromate and 0·2% osmium tetroxide varied between 4 and 8 days, and the silver nitrate treatment was 2–5 days. Specimens were embedded into Necoloidine (BDH) and serially sectioned at 80 μm. After dehydration, the sections were mounted in Depex (Gurr) using coverslips. To show nerve cells and their distribution in the optic tectum, drawings were made with the aid of a Zeiss camera lucida.

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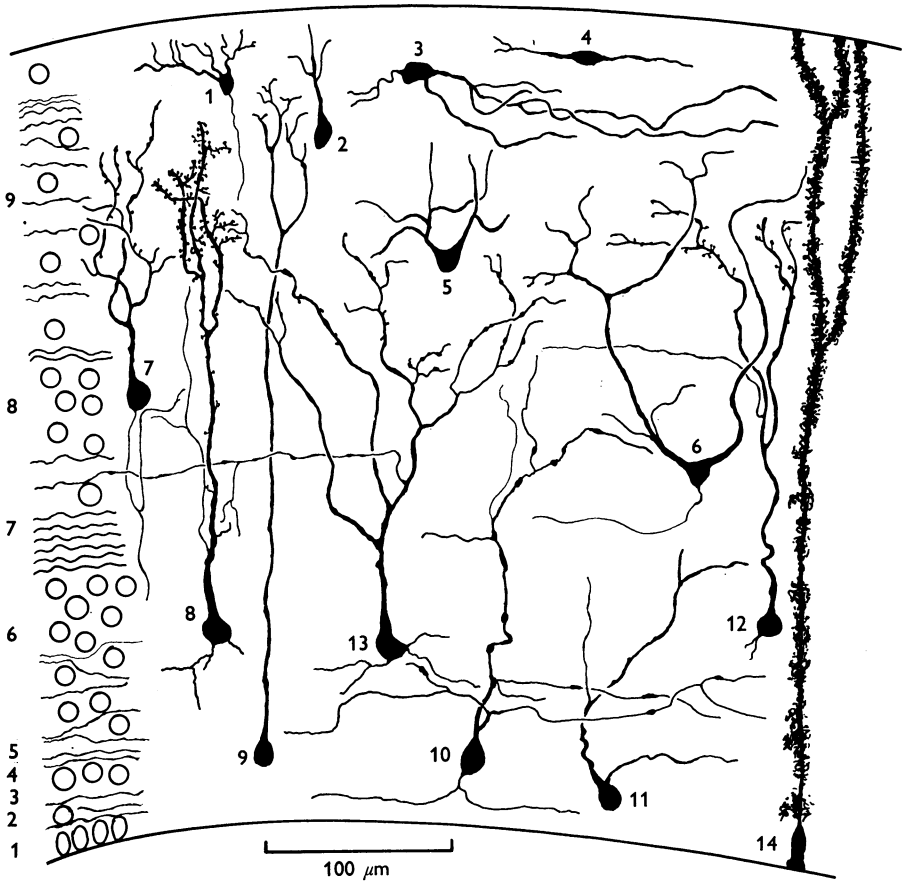

Fig. 1. Main cell types in the optic tectum of adult *Xenopus laevis*. Numbers on the left indicate tectal layers, which are shown schematically. Other numbers label neurons. 1, stellate; 2, amacrine; 3, horizontal; 4, fusiform or bipolar; 5, small ganglionic; 6, large ganglionic cells; 7, small pear-shaped cell with recurrent axon; 8, pear-shaped cell with ascending axon and narrow dendritic tree – note the presence of several spine-like dendritic appendages; 9, axonless pear-shaped cell; 10, 11, pear-shaped cells in the deep periventricular layers; 12, pear-shaped cell with intratectal axon running horizontally; 13, pyramidal cell; 14, ependymal cell. The cell processes drawn with the finest lines represent axons. Composite camera lucida drawing of coronal sections from rapid Golgi preparations.

Eighteen brains of adults, four brains of young toads and 107 tadpoles with acceptable or good impregnation in the tectum have been used in this study.

In the Golgi drawings, the numbers beside individual neurons refer back to the numbered neuron classes shown in Fig. 1.

RESULTS

The structure of the optic tectum in the adult *Xenopus laevis* is very similar to that of other anuran amphibians. Therefore it seems unnecessary to give a detailed description for *Xenopus* and the reader is referred to the literature (Ramon, 1890, 1946;

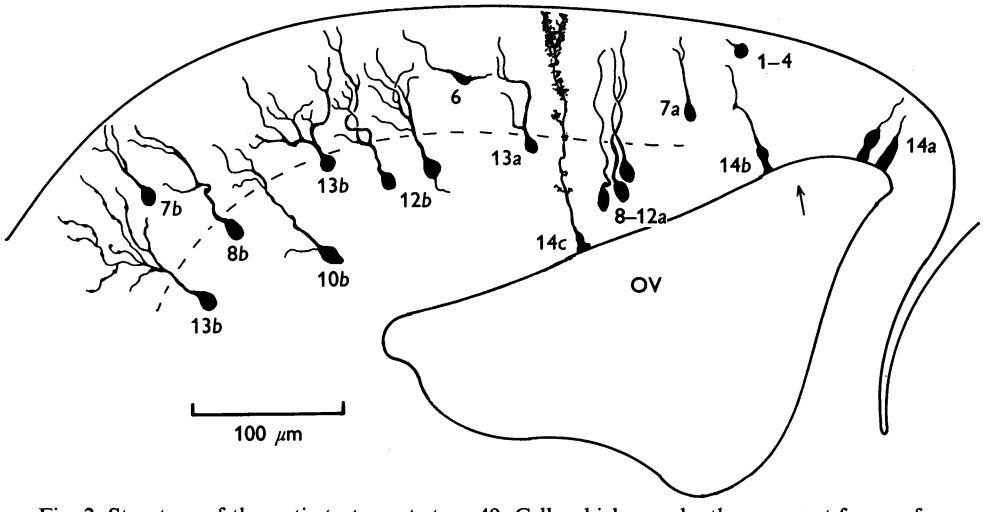

Fig. 2. Structure of the optic tectum at stage 49. Cells which may be the youngest forms of a particular neuron are labelled with the same numbers as in Fig. 1. *a* labels the younger, *b* and *c* the more advanced forms. The interrupted line represents the outer surface of layer 6. The arrow indicates the region of intensive cell division. OV, optic ventricle. Composite camera lucida drawing from two successive parasagittal sections of a rapid Golgi preparation. Rostral is to the left.

Kiro, 1948; Lázár & Székely, 1967; Potter, 1969). The main cell types, the development of which could be followed in the present investigation, are summarized in Fig. 1.

Differentiation of tectal neurons

The youngest tadpole to give successful impregnation of the tectum in the present series was at stage 49. At this stage the caudo-medial third of the tectum is a thin layer of undifferentiated neuroepithelial cells. The only elements in this region stained by the Golgi technique are very young ependymal cells with a smooth, slender process pointing towards the surface (Fig. 2: 14*a*). In the region of intensive cell division (Fig. 2, arrow), the ependymal cells have already one or two protrusions on their processes, and further rostrally they are almost completely developed (Fig. 2: 14*c*).

The youngest neurons to be impregnated are small cells localized just in front of the zone of cell division close to the surface of the tectum (Fig. 2: 1–4). These could be the ancestors of the types of neurons in layer 9 labelled 1–4 in Fig. 1.

The young cells labelled 8–12*a* in Fig. 2, which were impregnated in small groups, have single processes twisting around each other as they grow towards the surface. It seems likely that these are the precursors of the large pear-shaped neurons.

In the rostral half of the tectum the result of cell differentiation is obvious. From the main dendrites several side branches originate, and in addition to the neurons with a narrow dendritic tree, cells with wide dendritic arborization are present (Fig. 2: 13*b*). Some of them have beaded side branches which are very common in efferent neurons (Fig. 1: 13; Fig. 3: 13*b*). Basal dendrites are very rare at this stage; if present, they are no more than a single short process projecting from the ventricular pole of the cell body. These appear in large numbers after stages 51–52.

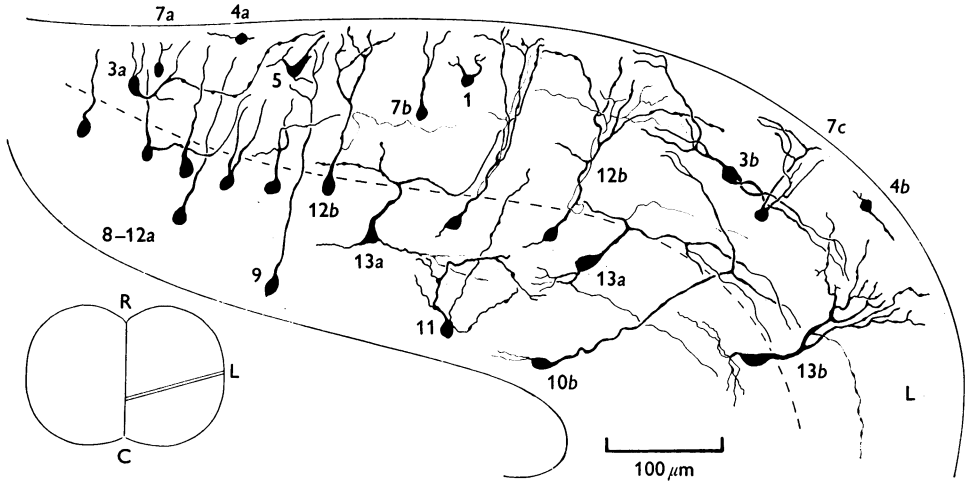

Fig. 3. Structure of the optic tectum of stages 55–56. Labelling is the same as before. The schematic drawing in the left lower corner shows the plane of reconstruction. R, rostral; C, caudal; L, lateral. Composite camera lucida drawing from rapid Golgi preparations.

The large ganglionic cells are very simple even in the middle third of the tectum at stage 49 (Fig. 2: 6), but one stage later, those localized in the rostro-lateral part of the tectum can have several long dendrites, as was found in one of the preparations.

By stages 55–56 the structure of the tectum becomes much more complicated. Figure 3 shows the progress of differentiation in the mediolateral direction. The youngest elements in the medial one-third are very similar to some found at stage 49 (Fig. 2). Further laterally, in the periventricular layers, basal dendrites appear, and efferent type cells at the lateral edge of the tectum represent the most advanced forms in these stages (Fig. 3: 13*b*). In the outer layers, cell differentiation results in the increased growth of cell processes in length and in the production of side-branches.

After stage 58, intensive cell division stops (Straznicky & Gaze, 1972), but differentiation goes on till metamorphosis, and is probably completed only in the juvenile toad. The most obvious changes in this period occur on the dendrites of pear-shaped cells with a narrow dendritic tree (Fig. 1: 8). These cells have a long apical dendrite, which gives origin to some branches in layer 9. These branches remain close to each other, so forming a cylindrical dendritic bush. In stage 51 (Fig. 4), the surface of these dendrites is relatively smooth with no protrusions on it. At stage 57, in a well-impregnated specimen, short, fine side branches and some spine-like processes could be observed on the dendrites (Fig. 5). These specializations are fairly common later than stage 60, and are usually well impregnated in the adults (Fig. 6). In parallel with this change, the basal dendrites grow in length and arborize, intermingling with each other and with the ingrowing nerve fibres in the deep plexiform layers (Figs. 7–9).

Development of the optic terminals

Stage 50 was the earliest stage at which optic terminals became impregnated in this material. In parasagittal sections of the brain, some well-stained optic fibres were found, arborizing in the outer part of the tectum (layer 9). The relatively thick

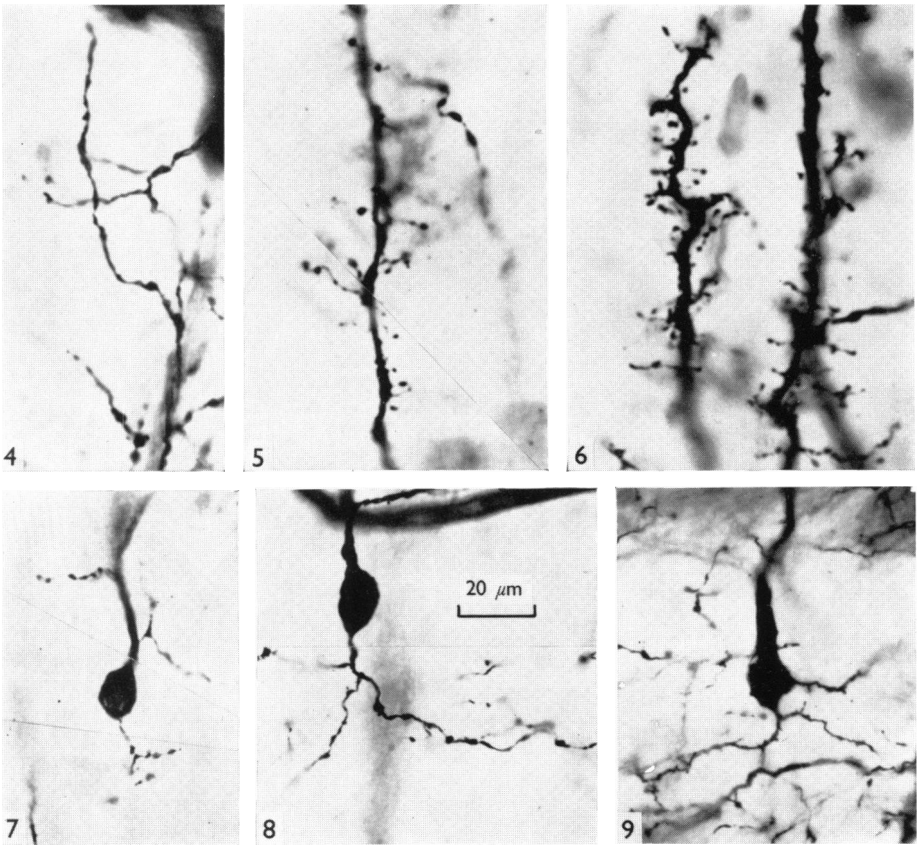

Fig. 4. Apical dendrite of a pear-shaped cell at stage 51. No dendritic specializations are present.

Fig. 5. Apical dendrite of a pear-shaped cell at stage 57. Note the presence of some dendritic appendages.

Fig. 6. Apical dendrite of a pear-shaped cell in adult *Xenopus laevis*. Several dendritic appendages are impregnated.

Fig. 7. Basal dendrite of a pear-shaped cell at stage 51.

Fig. 8. Basal dendrites of a pear-shaped cell at stage 57. The scale on this figure applies to Figs. 4–9.

Fig. 9. Basal dendrites of a pear-shaped cell in adult *Xenopus laevis*. Rapid Golgi technique.

parent fibre divides into two or three main branches, and these are the sources of finer end twigs. The terminal is flat but rather elongated, measuring 20–30 μm dorsoventrally and 150–180 μm in length.

At stage 57, similar terminals were stained. The length of one well-impregnated terminal approached 300 μm, but relatively few secondary/or tertiary branches originated from the stem fibres.

The optic terminals in adults show the same characteristic features that have been described by Potter (1972) in *Rana catesbiana* and Székely, Sétáló & Lázár (1973) in *Rana esculenta*. As in the other species, the thick parent fibres give rise to finer

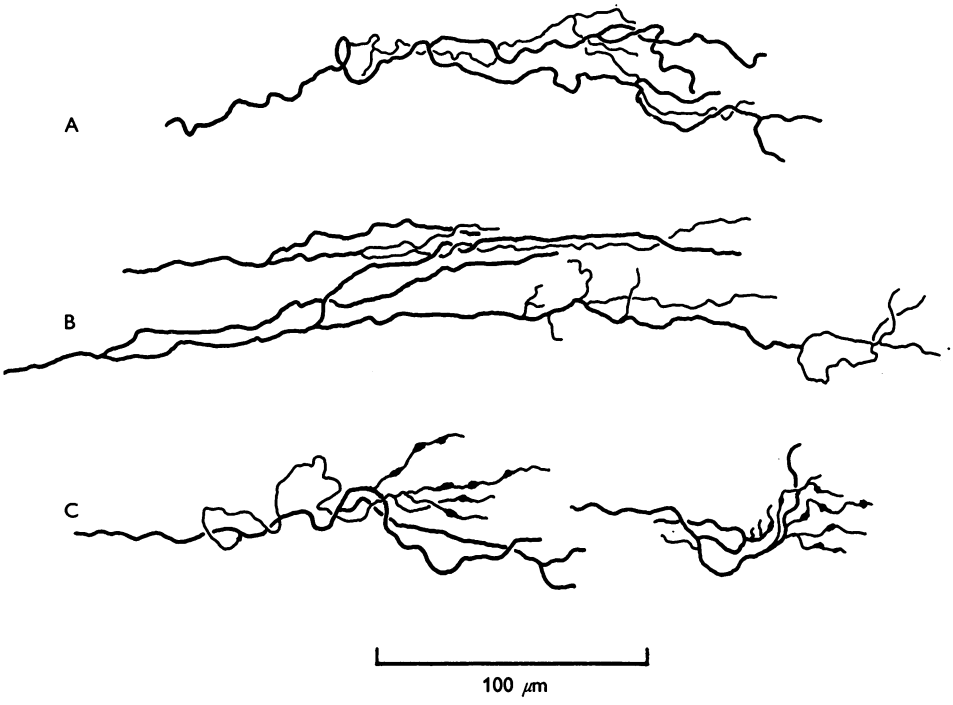

Fig. 10. Optic terminals in the tectum. A, stage 50; B, stage 57; C, adult. Camera lucida drawings of parasagittal sections from rapid Golgi preparations. Rostral is to the left.

branches, the majority of which are beaded. The length of the terminal bush does not exceed 150 μm. Only the outer half of layer 9 was relatively well impregnated in our preparations, so we have no information about deeper optic terminals.

DISCUSSION

In a short description of the tectal structure in larvae of *Hyla regilla*, Larsell (1929) mentioned that the caudal part of the tectum was less differentiated than the rostral half. According to the present investigations, differentiation seems to be a relatively simple process in the case of neurons in layer 9, and the large ganglionic cells. As the cell becomes left behind by the caudalwards movement of the mitotic region, one or more processes emerge from the cell body and these produce side branches and grow in length to reach their final shape and size. We do not know at which stage this process can be regarded as complete.

The differentiation process appears to be more complex in pear-shaped cells and it is possible to distinguish three phases in their life. In the first phase, younger than stage 50, the apical dendrite develops intensively, producing side branches of various lengths and shapes, which can be smooth or beaded. In the second phase, between stages 50 and 57, the basal dendrites develop and reach a considerable length in those neurons with a small apical dendrite (Fig. 1: 10). Efferent type cells, which have a widely arborizing dendritic tree, usually have several long basal dendrites also.

It is probable that most of the axons are produced in this phase. Axons were not stained at stage 49. Naturally, this could be the consequence of an insufficient impregnation, but they appeared in increasing numbers at later stages and, by stage 55, axons of different kinds were frequently seen in well-impregnated preparations.

The third phase in the differentiation of pear-shaped neurons is marked by the appearance of spine-like appendages on the apical dendrites in increasing numbers after stage 57; the appendages, however, only complete their development after metamorphosis. These changes are characteristic of the cells labelled 8 and 12 on Fig. 1, and are very similar to those described by Cajal (1960) for cortical pyramidal cells.

The progress of development of individual tectal cells has been shown above in reference to the time elapsed after the formation of a particular cell. Since cells are not at the same age in the caudal and the rostral part of the tectum, progressive differentiation is recognizable according to the position of a cell within the tectum. This means that the simplest neurons are localized always in the caudo-medial part, and the most advanced forms in the rostro-lateral region of the tectum until development is completed.

This peculiar structure of the developing tectum raises the question of how the most recent physiological data can be correlated with structural changes during development. To attempt to answer this question, we would need more detailed knowledge about tectal structure, especially about optic terminals. I realize that the information gained in this study on optic terminals is far from sufficient, because only a few terminals were impregnated either in larvae or in adults, and it is uncertain whether the whole terminal bush was stained. However, if the terminals of optic fibres are compared in a younger and older tadpole, or these are compared with adult terminals, some differences, which seem to be consistent, can be recognized.

The optic terminals were always bigger in tadpoles than in adults. At stage 50, about one-third of the available space was covered by a single optic terminal in layer 9 in the parasagittal plane. This proportion was about one-fourth in stage 57, and only one-tenth in the adult; and the absolute length of the terminal was also reduced in adults. This gradual diminution in the size of the area covered by a single optic fibre could be the cause of the reduction in the size of multi-unit receptive fields during development (Gaze *et al.* 1972).

If the thickness of the area covered by an optic terminal in the vertical plane is compared with the thickness of the tectum above layer 6, we find that the proportion of terminals to outer tectal layers is approximately 1:3 at stage 50, 1:4·5 at stage 57, and 1:6 in the adult. Comparing the Golgi preparation to a section stained by the Nissl technique, it is very easy to see that one optic terminal can cover more than half of the thickness of layer 9 at stage 50, which results in extensive overlap in depth between adjacent terminals. A considerable overlap exists at stage 57 as well, but optic terminal layers are rather well separated in adults. These morphological data agree with electrophysiological observations, according to which the projection of the different retinal detectors cannot be separated from one another before stage 60 (Chung *et al.* 1973).

The last point to be discussed is the possible relationship between dendritic appendages and optic terminals. It was shown in an electron microscopic study

(Székely *et al.* 1973) that dendritic appendages are in synaptic contact with optic terminals in adult *Rana esculenta*. It seems reasonable to assume that the situation is similar in the *Xenopus* tectum. Synapses between optic fibres and tectal dendrites are already present in early larval stages (T. M. Scott, personal communication), when the cells are immature and the optic terminals are big. As development continues, more and more synaptic surface is provided by a single cell for the optic fibres. In parallel with this, the optic fibres have to produce more and more side branches, or to make multiple connexions. By the time the dendritic appendages develop fully, the optic terminals seem to be reduced in size. It is highly probable that these changes involve disappearance of pre-existing synapses and the establishment of new synaptic linkages. These findings seem to support the hypothesis put forward by Gaze *et al.* (1972) that the transposition of the projection of the visual field during development is the consequence of a caudalward shift of retinotectal connexions.

SUMMARY

The development of the optic tectum has been studied by the rapid Golgi technique.

The process of cell differentiation in certain pear-shaped cells involved the appearance of spine-like dendritic appendages on the apical dendrites in increasing numbers, and the emergence of basal dendrites from the cell body.

During development the less differentiated neurons are always localized in the caudo-medial part of the tectum.

The proportion between the size of optic terminals and the area covered in the tectum decreases towards the metamorphic climax.

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