# Precocious retina development caused by receptor cell independent light effect

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*Summary.* In the retina of the mouth-brooding teleost *Tilapia leucosticta*, light causes an accelerated development of ganglion cells. This effect is presumably not mediated by receptor and interneuronal cells.

An influence of dark rearing on retina development is either absent[1], or results in a reduction[2] or a delay of maturation[3]. Correspondingly, light effect has been shown to be either stimulating[4] or accelerating[5]. The latter reports, however, are confined to evoked responses without reference to structural differentiation of the retina. So far, all attempts to produce an effect on eyes which normally develop in the darkness, by placing them into a lighted environment, seem to have failed[6].

In the present study, an influence of light on retinal ganglion cells will be shown, which normally develop under very dim light conditions. This influence is probably independent of receptor and interneuronal cells, and causes a precocious but ordered development of certain retinal structures.

The studies have been performed on *Tilapia leucosticta*, a mouth-brooding teleost. The development normally takes place in the relative darkness of the mother's mouth. The experimental conditions consisted of placing the embryos into a brooding apparatus with either continuous light (L; 6 40-W fluorescent lamps in a room of 18 $m^3$) or continuous darkness (D; same room, but covered by a box, impenetrable for light) from the 3rd day after fertilization up to the day of sacrifice. Embryos of both L- and D-series were reared wholly alike, sharing water and room conditions except for light supply. Specimens were fixed every 24 h up to day 13, the date they normally leave their mother's mouth. Dark reared animals were fixed in total darkness. For light microscopy, entire heads were fixed in Carnoy's fluid and stained by varying techniques. For electron microscopy, retinae were excised and fixed in glutaraldehyde, followed by either osmic acid or ethanolic phosphotungstic acid[7], and embedded in Maraglas. Experiments were repeated several times with embryos of different parents.

Table 1. Ganglion cell number/200 $\mu m^2$ (*A*), tractus opticus diameter as determined by the number of sections on which it occurs when leaving the retina (*B*), inner plexiform layer thickness in $\mu m$ (*C*) and their changes with advancing age and under light (L) and darkness (D) conditions

| | Day | L | D | p≤ |
|---|---|---|---|---|
| *A* | 6 | 6.8 ± 0.6 | 6.2 ± 0.5 | |
| | 7 | 6.2 ± 0.4 | 6.1 ± 0.6 | |
| | 8 | 5.3 ± 0.4 | 6.6 ± 0.5 | 0.001 |
| | 9 | 4.9 ± 0.5 | 5.6 ± 0.6 | 0.001 |
| | 10 | 4.4 ± 0.5 | 6.8 ± 0.4 | 0.001 |
| | 11 | 4.2 ± 0.6 | 6.2 ± 0.8 | 0.001 |
| | 12 | 4.6 ± 0.5 | 6.8 ± 0.6 | 0.001 |
| | 13 | 4.5 ± 0.7 | 5.8 ± 0.5 | 0.001 |
| *B* | 6 | 31.4 ± 3.1 | 26.8 ± 2.3 | 0.05 |
| | 7 | 38.0 ± 3.6 | 34.7 ± 3.5 | |
| | 8 | 43.3 ± 2.5 | 39.1 ± 4.8 | |
| | 9 | 45.5 ± 4.4 | 38.8 ± 4.0 | 0.02 |
| | 10 | 53.3 ± 7.2 | 37.9 ± 5.7 | 0.001 |
| | 11 | 51.5 ± 2.9 | 38.0 ± 3.4 | 0.001 |
| | 12 | 50.4 ± 1.9 | 38.7 ± 4.6 | 0.01 |
| | 13 | 50.0 ± 3.5 | 45.2 ± 6.6 | |
| *C* | 6 | 12.9 ± 0.8 | 10.7 ± 1.1 | |
| | 7 | 15.5 ± 1.4 | 14.5 ± 0.5 | |
| | 8 | 16.4 ± 1.8 | 16.4 ± 1.1 | |
| | 9 | 21.1 ± 0.8 | 16.7 ± 1.2 | 0.001 |
| | 10 | 20.1 ± 1.6 | 18.7 ± 0.8 | |
| | 11 | 22.8 ± 2.1 | 17.1 ± 0.8 | 0.05 |
| | 12 | 25.1 ± 1.0 | 18.0 ± 0.6 | 0.001 |
| | 13 | 24.4 ± 2.1 | 21.1 ± 1.5 | |

Countings and measurements have been performed with the light microscope on 5 $\mu m$ paraffin sections using an object micrometer and an ocular grid. Only the most advanced central part of the retina has been taken into account. p-values are given only when ≤ 0.05.

Table 1 shows the changes in a) number of ganglion cells per 200 $\mu m^2$, b) the tractus opticus diameter and c) the thickness of the inner plexiform layer, with advancing age and under L- and D-conditions. From day 7 on, ganglion cell density in the L-retina decreases steadily up to day 10, while in the D-retina it is found to remain rather constant. Significant differences (Student's t-test, $p \leq 0.001$) result for days 9 through 13. The diameter of the tractus opticus (and with that, the number of ganglion cell axons) increases steadily from day 3 on, and already beginning with day 6, the D-tractus remains a little bit smaller than the L-tractus. Starting with day 9, its increase stops transitorily while the L-tractus continues to grow and after day 9 attains a level which is attained by the D-tractus only after day 13. Significant differences ($p \leq 0.001$) were found on days 10 and 11. The thickness of the inner plexiform layer increases in the D-retina only at a reduced rate after day 8, at which date the greatest increase occurs in the L-retina. Already before day 9, the inner plexiform layer in the D-retina is constantly lower than in the L-retina, and after day 12 the differences tend to equalize. In table 2, the results of synaptic junction countings in the inner plexiform layer are summarized. In the D-retina, first synaptic junctions appear at the end of day 7; they are probably not mature, but they do not increase essentially before day 11. In the L-series they make their appearance already on day 6 and increase continuously on the following days. Significant differences between L and D are found only on days 9 and 10. Synaptic sites with a ribbon appear for the first time in an immature state on day 7 in either series and show a similar development

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Table 2. Number of synaptic junctions of conventional (*A*) or ribbon (*B*) type with advancing age and under light (L) and darkness (D) conditions

| | Day | L | D | p⩽ |
|---|---|---|---|---|
| *A* | 6 | (0.2) | – | |
| | 7 | (0.1) | (1.1) | |
| | 8 | 2.2 ± 0.8 | 1.0 ± 0.2 | |
| | 9 | 5.2 ± 1.9 | 1.2 ± 0.4 | 0.02 |
| | 10 | 4.2 ± 0.3 | 1.1 ± 0.3 | 0.001 |
| | 11 | 5.3 ± 0.6 | 2.8 ± 0.9 | |
| | 12 | (4.6) | 4.3 ± 2.1 | |
| | 13 | 5.5 ± 0.7 | 4.1 ± 1.4 | |
| *B* | 6 | – | – | |
| | 7 | 0.2 | 0.1 | |
| | 8 | 0.3 ± 0.1 | 0.7 ± 0.4 | |
| | 9 | 0.6 ± 0.2 | 0.7 ± 0.3 | |
| | 10 | 0.8 ± 0.3 | 0.2 ± 0.1 | |
| | 11 | 1.5 ± 0.1 | 0.5 ± 0.1 | 0.01 |
| | 12 | (1.8) | 1.0 ± 0.6 | |
| | 13 | 1.1 ± 0.4 | 1.8 ± 0.6 | |

Junctions have been counted over the greatest possible area in the central part of the retina on E-PTA stained sections[7], and calculated to 100 $\mu m^2$. Before day 8, the number of junctions is very low and no statistical operation was possible. For day 12, L-values are incomplete. p-values are only given when ⩽ 0.05.

with the exception of a sharp regression in the D-retina on day 10. The counting of synaptic vesicles in ultrathin sections of axon terminals revealed 3 groups of terminals; with <20, 20–50 and >50 vesicles per terminal section (details will be given in another paper in preparation). In the D-series, until day 7 only terminals with less than 20 vesicles per section were found. Terminals with more than 20 and more than 50 vesicles appeared on days 8 and 10 respectively. In the L-retina, terminals with 20–50 vesicles are represented already on day 6, those with more than 50 on day 7. Dense core vesicles of the inner plexiform layer do not occur before day 10 in the D-retina, when they are found in 6% of all terminals. This percentage will not change up to day 13. In the L-retina they appear already on day 8, account for 8% on day 10 and for 10% on day 12.

In the retina of embryos and larvae reared in light conditions, we observe at about day 8 an increase in the number of ganglion cell axons, thus augmenting tractus opticus thickness and lowering the number of ganglion cells per area, along with an increase of number and ramifications of ganglion cell dendrites. This latter increase may stimulate the formation of synaptic contacts and of synaptic vesicles and ribbons in interneuronal processes. In the D-retina, all this occurs some 2 or 3 days later, when the values for the L-retina are attained. The net increase for inner plexiform layer thickness, tractus opticus diameter and synaptic junction number between days 6 and 13 is nearly the same in both L- and D-series. Therefore one has to assume that continuous light, being the experimental situation for a normally dark reared animal, induces a precocious rather than a simply stimulated growth of retinal structures. The sequence of developmental processes seems not to be altered, and neither light nor darkness effects a disordered development.

At the time when first differences between L- and D-retinae arise, receptive and transmitting structures of the retina just start their development. Receptor cell synaptic vesicles and outer segment membranes appear on day 6[8], but it is doubtful whether the latter are capable of receiving light impulses at this date[9]. In the inner plexiform layer, first synaptic junctions are seen on day 7 and they are probably not mature before day 8, that is to say synchronously to first expressions of light-darkness-differences. Thus, the light seems to exert its effect not via the receptor cells, but directly onto the ganglion cells. There are several examples of a nonvisually mediated photosensitivity on developmental stages[10], presumably connected to hormonal action. It is, however, noteworthy that Hansson and Sourander[11] described a pronounced sensitivity to light of retinal ganglion cells in vitro. The question whether the effect is mediated by receptor cells of retina or pineal organ, or by the tectum opticum, is presently under study.

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## Histochemical and ultrastructural modifications of mice endometrium, vagina and pituitary following zeranol treatment

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*Summary.* The histochemical and ultrastructural changes produced by zeranol treatment on the endometrium and the gonadotropic FSH cells of the adenohypophysis have been investigated in mice at the prepuberal and virginal stages. Modifications similar to those induced by estrogen treatment were observed. It is concluded that both estrogens and zeranol share the same activity on the tissues examined.

The aim of this study was to investigate the effect of a new compound, obtained from cultures of molds *Fusarium graminearum*[1] and used in clinical practice for treatment of menopausal symptoms[2], on the histology of mice ovaries and pituitary.

Mice at the prepuberal and virginal stages were injected i.p. with 50μg and 100μg of zeranol 6 (6–10-dehydroxyundecil) β-resorcylic acid μ lactone supplied by the Istituto Chemioterapico Italiano daily for a period of 7 days. Tissue slices from uterus, ovary, vagina and