The Effect of Two Forms of Light on the Development of the Neonatal Rat Retina

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Summary. The effects of red and far red light on the development of the retinas were studied in the neonatal rat models. Red light appeared to be stimulatory and far red light appeared to be inhibitory.

Embryologically, vertebrate retinas were found to develop normally even in the total absence of light[1], whereas, at the other extreme, excessive illumination would result in the decrease in thickness of the outer retinal layers in the neonatal rat[2]. Does light exert a stimulatory or inhibitory effect on the developing system? And, do all wavelengths in the visible light spectrum exert the same or different effects on this system? In order to shed some light on the problem, we chose 2 forms of light (red, $\lambda = 660$ nm and far red, $\lambda = 730$ nm) to study their effects on the developing rat retinas. The neonatal rats were chosen because their visual cells developed in the neonatal period[3].

Material and methods. 7-day-old neonatal rats (strain: Simonsen) were selected for the experiment. The rats were divided into 3 divisions, the red (R), far red (FR) and the controls. The animals of the first 2 divisions were subdivided into 3 groups, e.g. R_1, R_2 and R_3, each subjected to different days of special light illumination. Inside each group, the animals were again divided into 3 subgroups, e.g. R_{II}, R_{III} and R_{IIII}, each subjected to different durations of treatment. A detail schedule was set up as in Table I. The intensity of red light illumination was 200 watt cm^{-2}, and the intensity of far red light illumination was 50 watt cm^{-2}. It is essential to point out that the experimental animals were given their red or far red light treatment along with the usual daylight-dark cycle. The experimental animals of each subgroup were compared with controls which came from the same mother so as to eliminate any genetic variation.

After the treatments, both experimental and control animals were killed and their eyeballs taken out. These were then fixed in Bouin, dehydrated, cleared and embedded in paraffin. The eyes were then cross sectioned at right angle to the longitudinal axis at 6 μm thickness and the 20th sections passed the lens were selected for observation, thus ensuring almost identical regions were used. The sections were then stained with Hematoxylin and Eosin. The thickness of the outer segments and the thickness of the retinas were measured with the aid of an ocular and stage micrometer under the same magnifications. The ratios of the thickness of the outer segments to the thickness of the whole retinas were then calculated.

Results. Histological observations on the retinas of both treated and control animals revealed a significant difference in the ratios of the thickness of the visual layers to the total thickness of the retinas (Figures 1–3).

The results of careful morphometric study on the ratios of the thickness of outer segments to the thickness of the retinas were illustrated in Tables II–IV.

It appeared that red light stimulated the growth of the outer segments of all experimental groups except R_3 subgroup II and R_1 and R_2 subgroups III. Far red light, on the other hand, would inhibit the growth of all outer segments when compared with the control animals.

Discussion. The effect of light takes many forms in the visual system of the mammals. It is responsible for the changes of both rhodopsin and catecholamine[4]. Excessive light, however, will cause a decrease in thickness of the outer retinal layers[2].

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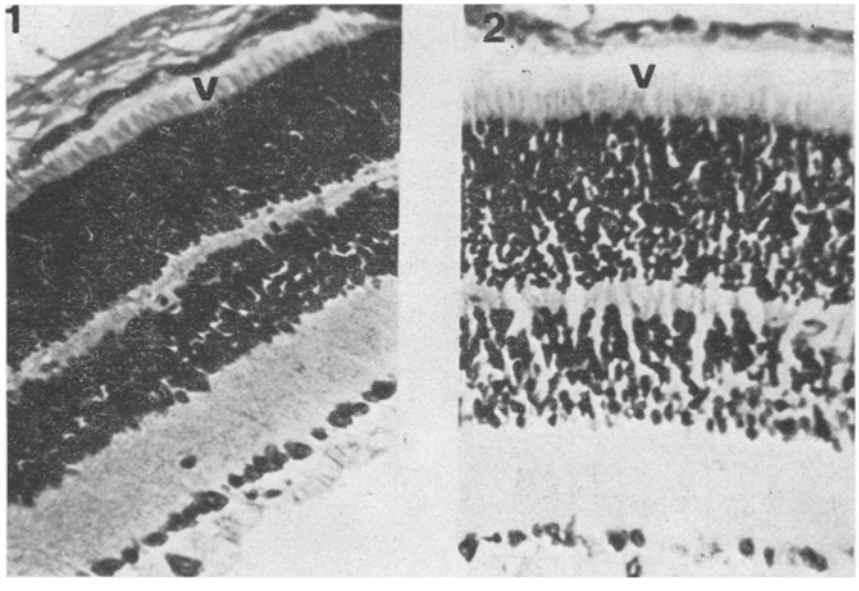

Fig. 1. Light micrograph of a normal 14-day-old neonatal rat retina. Note the thickness of the visual layer (V) and the thickness of the retina. ×450.

Fig. 2. Light micrograph of a 14-day-old neonatal rat retina after red light treatment. Note increase in thickness of the visual layer (V). ×450.

Table I. Schedule of light treatment for different groups

Type of light		Red			Far red			Control
Groups		R_1	R_2	R_3	FR_1	FR_2	FR_3	
Days of light treatment		7–13[a]	7, 9, 11, 13	7, 10, 13	7–13	7, 9, 11, 13	7, 10, 13	Ordinary daylight-dark cycle
Duration of light treatment eachtime (min)	Subgroup I	30	30	30	15	15	15	
	Subgroup II	20	20	20	10	10	10	
	Subgroup III	10	10	—	5	5	—	

[a] Postnatal day.

Table II. Results of differences in Ratio of $\frac{\text{outer segment thickness}}{\text{total retinal thickness}}$ between control and experimental subgroup I

Subgroup	$\bar{X}_1$	$\bar{X}_1-\bar{X}_2$	Probability from *t*-test	Difference between control and experimental significant or not
R_1 subgroup I	0.0283	+ 0.0038	0.05	significant
R_2 subgroup II	0.0310	+ 0.0065	0.001	significant
R_3 subgroup III	0.0468	+ 0.0223	0.001	significant
FR_1 subgroup I	0.0209	− 0.0036	0.02	significant
FR_2 subgroup II	0.0178	− 0.0067	0.01	significant
FR_3 subgroup III	0.0133	− 0 0112	0.001	significant

$\bar{X}_2$ = mean ratio of $\frac{\text{outer segment thickness}}{\text{total retinal thickness}}$ of control.

$\bar{X}_2$ = mean ratio of $\frac{\text{outer segment thickness}}{\text{total retinal thickness}}$ of experimental.

$\bar{X}_2 = 0.0245$.

Table III. Results of difference in ratio of $\frac{\text{outer segment thickness}}{\text{total retinal thickness}}$ between control and experimental subgroup II

Subgroup	$\bar{X}_1$	$\bar{X}_1-\bar{X}_2$	Probability from *t*-test	Difference between control and experimental significant or not
R_1 subgroup II	0.0387	+ 0.0020	0.05	significant
R_2 subgroup II	0.0457	+ 0.0090	0.001	significant
R_3 subgroup II	0.0354	− 0.0013	0.05	not significant (reverse effect)
FR_1 subgroup II	0.0229	− 0.0138	0.001	significant
FR_2 subgroup II	0.0185	− 0.0182	0.001	significant
FR_3 subgroup II	0.0227	− 0.0140	0.001	significant

$\bar{X}_2$ and $\bar{X}_1$ same as in Table II. $\bar{X}_2 = 0.0367$.

Table IV. Results of difference in ratio $\frac{\text{outer segment thickness}}{\text{total retinal thickness}}$ between control and experimental subgroup III

Subgroup	$\bar{X}_1$	$\bar{X}_1-\bar{X}_2$	Probability from *t*-test	Difference between control and experimental significant or not
R_1 subgroup III	0.0249	− 0.0073	0.001	significant
R_2 subgroup III	0.0219	− 0.0103	0.001	significant
FR_1 subgroup III	0.0115	− 0.0207	0.001	significant
FR_2 subgroup III	0.0161	− 0.0161	0.001	significant

$\bar{X}_2$ and $\bar{X}_1$ same as Table II. $\bar{X}_2 = 0.0322$.

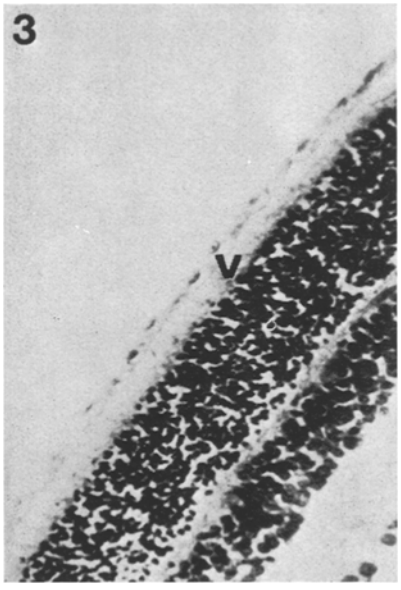

Fig. 3. Light micrograph of a 14-day-old neonatal rat retina after far red light treatment. Note decrease in thickness of the visual layer (V). ×450.

Our data indicate that light affects the development of the mammalian retinas as well, but perhaps in many different ways. Two closely related light waves (red and far red) exert totally different effects in the developing visual system. Red light appears to be stimulatory and far red light appears to be inhibitory. Since our experimental R_3 subgroup II (only treated with red light for once every 3 days) and R_1 and R_2 subgroups III (only treated with red light for 10 min each time) did not show any stimulatory effects after the red light treatment; therefore it seems that both timing and duration of treatment are critical factors. There is probably an existing minimum dosage for the treatment to be effective.

The mechanism of how 2 forms of light affect the retina, in particular the outer segments, remains obscure at this stage. A possible first target is perhaps the myoid region of the inner segments, in which groups of RNA are located. Since there is the myoid RNA that is responsible for the regeneration of the outer segments[5], it is reasonable to predict that an increase or decrease in thickness of the outer segments will result if one monitors the amount of RNA in this region. It could be that the light treatment affects directly the myoid region of the inner segments and causes an effect on the outer segments.

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Morphological Evidence for Axonal Transport of Glycogen in Neurons Innervating Cutaneous Receptors in *Lacerta sicula* (Squamata: Reptilia)

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Summary. Three types of glycogen-containing cutaneous nerve terminals – two of them hitherto unknown – are described. It is shown that the glycogen is synthesized in the perikaryon and transferred to the terminals by means of slow axoplasmic transport.

There are several types of nerve terminals in the skin of *Lacerta sicula* containing glycogen (Figures 4, 5 and 6). Since the terminals show no organelles for glycogen-synthesis, the question arizes as to how glycogen gets to them. As shown below, it appears very probable that it is brought to the terminals by axonal transport.

Wanson and Drochmans[1] showed that, in the skeletal muscle fibre of the rabbit, glycogen β-particles are being synthesized on sarcoplasmic vesicles (derivates of the sarcoplasmic reticulum). As far as we know, this fact has not been confirmed for nerve cells. Berthold[2] found that, in spinal ganglia cells of the frog, aggregations of glycogen particles often lie close to smooth surfaced membrane systems. This may be interpreted as implying that in neurons also glycogen is synthesized on membranes of the smooth endoplasmic reticulum. Observations made by Ibrahim[3], using enzyme-histochemical methods, also support the hypothesis of glycogen-synthesis in the perikaryon.

Glycogen synthesized in the perikaryon is transferred to the terminals by axoplasmic transport. Morphological proof for this hypothesis is given by the following observations: 1. In the lower regions of the corium, myelinated axons densely packed with glycogen are found (Figure 1). These axons contain mitochondria, vesicles and – depending on the type of fixation and anesthetic used – neurofilaments and neurotubules. The glycogen-containing sections of the axon are somewhat wider in diameter. 2. In the superficial corium, the axons after having lost their myelin lamellae show bead-like protrusions (Figure 2) which contain – in addition to large amounts of glycogen – mitochondria, neurofilaments, neurotubules and vesicles. These 'beads' are always covered by a continuous Schwann-cell sheath. 3. 'Beads' similar to those mentioned above can be found in the intercellular space of the epidermis (Figure 3). Since the Schwann-cell sheath ends at the basal lamina, neither the beads nor the axons are surrounded by it.

The presence of 'beads' in an axon is due to the discontinuous transport of glycogen. Since discontinuous transport is found only in slow axoplasmic transport[4], it can be concluded that glycogen is transferred to the terminals be means of slow axoplasmic transport.

Among the glycogen-containing terminals, the following types can be distinguished: 1. A terminal portion (with its finger-like protrusions) of the receptor axon in the non-encapsulated lamellated corpuscles in the corium

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