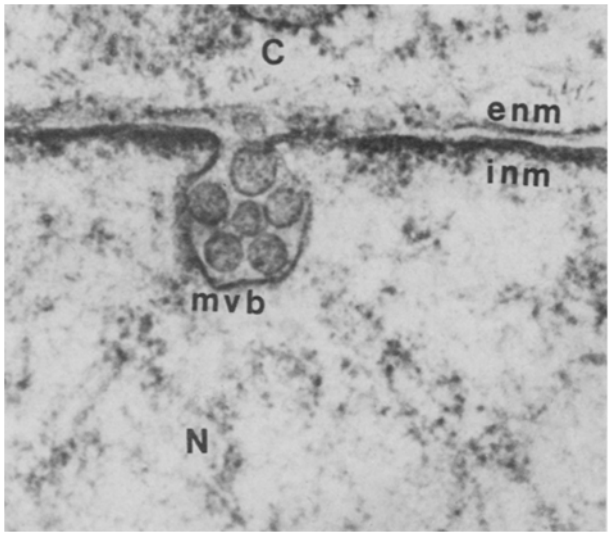

Fig. 1. A multivesicular body containing 6 vesicles is clearly seen as an infolding of the internal nuclear membrane. Note continuity of the MVB membrane with the INM. The external nuclear membrane is apparently not involved in the formation of the nuclear MVB; N, nucleus; C, cytoplasm; INM, internal nuclear membrane; ENM, external nuclear membrane. × 69,000.

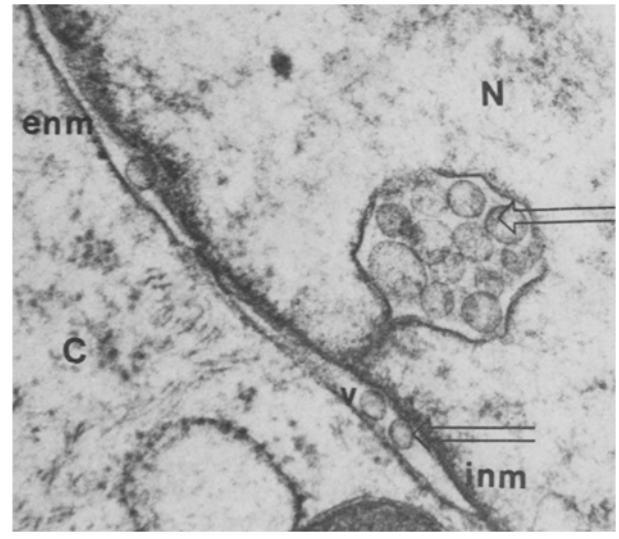

Fig. 2. Small vesicles (V) are found to lie free between the internal INM and external nuclear membranes (ENM). Note similarity between these vesicles and those filling the MVB (arrows). The vanishing and elongated aspect of the neck of the MVB suggests a possible separation of this structure from the nuclear membrane. × 66,000.

rous vesicles or tubules (Figure 1). We have observed that, in some of these structures, the wide connection between the baglet and the intermembranous space narrows into a very thin neck (Figure 2).

The external nuclear membrane bridges over the neck of the INM, invagination without taking part in the process. The small vesicles inside these structures are approximately 90 nm in diameter and they are surrounded by a single membrane, probably originating in the INM. We refer to these vesicular invaginations as MVB due to the morphological similarities with the well described cytoplasmic organelles.

Furthermore we noted nuclear MVB apparently unconnected with the INM. In addition to and sometimes in association with MVB, several single-membraned small vesicles appeared between the internal and external nuclear membrane (Figure 2). These vesicles were found to lie free in the intermembranous space, and although no connection with the nuclear membranes was noted, their similarity with vesicles of multivesicular bodies suggests their probable nuclear membrane origin.

The formation mechanism of these nuclear MVB might be very similar to the one described by KILARSKI and JASINSKI[7] (see figure legends). But, as only the internal nuclear membrane of BHK cells is involved, the development and possible separation of MVB from the INM could be more easily achieved.

As to the formation and nature of MVB, there are numerous reports relating these organelles to lysosomes, digestive vacuoles, Golgi system, and pinocytic vesicles[8–12]. In BHK cells, MVB are closely linked to the nuclear membrane and might be closely related to the intranuclear canaliculi described in many different tissues[15–17]. Furthermore their function culd be similar to those ascribed to the canalicular system, i.e.: transport of nuclear and nucleolar products of the cytoplasm and vice versa.

Zusammenfassung. Mit elektronenmikroskopischer Untersuchung von BHK 21 cl 13 Zellen wurden multivesikuläre Körperchen im Karyoplasma festgestellt, Strukturen, die direkt der inneren Kernmembran entstammen. Die multivesikulären Körperchen wie auch die internen vesikulären Elemente (⌀ 90 nm) waren von einer einzigen Membran umgeben.

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Effect of Light on the Synaptic Organization of the Inner Plexiform Layer of the Retina in Albino Rats

Visual receptors of albino rats are damaged by prolonged illumination[1–5]. In rhesus monkeys degeneration of the receptors in the macular region is observed after the eye has been exposed to the light of an indirect ophthalmoscope[6]. The extent of damage increases in severity with the length and intensity of illumination. When studying the effects of monocular deprivation (achieved by unilateral lid-suture) of pattern vision in

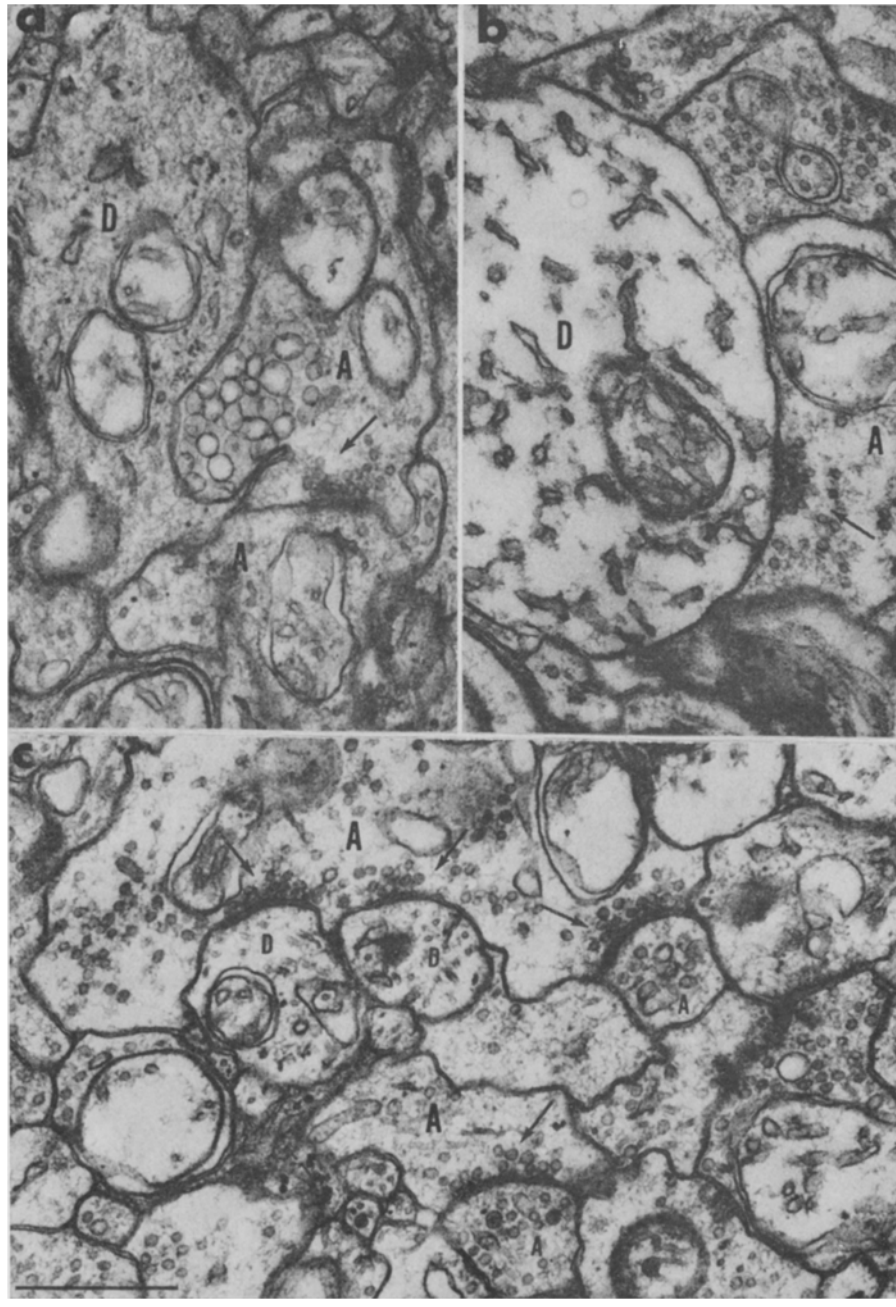

Fig. 1. Changes in dendritic profiles and amacrine endings (a, b) after long illumination (longer than 8 h per day for more than 2 months) as compared with controls illuminated 8 h for 2 months (c). In a) the dendrite (D) seems to be dark, the amacrine ending (A) contains vacuoles. In b) the dendrite (D) is swollen. The amacrine ending (A) contacting it seems to be normal. The altered structures retain their synaptic contacts. The presynaptic sides are marked by arrows. Calibration line 0.5 μm.

albino rats, damage to the receptors of the open eye, companion to the lid-sutured, was observed. In addition to the receptor damage, a decreased density of synapses of bipolar cells occurred[7,8]. It was postulated that a rat with only one functional eye keeps it open for a longer period of time than it would if both eyes were available. An increased illumination of this open eye would primarily affect receptors and consequently the synaptic endings of bipolar cells. The aim of the reported experiments was to test this assumption.

Material and methods. Starting on the 14th day of age albino rats were illuminated daily for 8 or 16 h for 2, 4 or 6 months with light of 500 lux intensity. The animals were kept at 24 °C on the usual Larsen diet. Under urethane anaesthesia they were sacrificed by perfusion with glutaraldehyde and the retinae were prepared for light and electron microscope examination as described elsewhere[7,8]. The thickness of various retinal layers was measured at a magnification of 1,000 × and the density of synapses counted in electron micrographs taken from the whole thickness of the inner plexiform layer (IPL)[8] in fields of 56.2 μm^2 at a magnification of 26,600 ×.

Results. Light microscopy. The retinae of rats illuminated for a relatively short period (8 h daily for 2 months)

Group		Thickness (μm)		Density of synapses per field*, per μm²**					Surface of IPL (μm²)
		Outer		Inner Plexiform Layer					
		Plexiform layer	Nuclear layer	Total	Dyad	A/B	A/A	A/D	
I	A (3) 8 h/2 mo	9.3 ± 0.14	49.1 ± 0.42	7.42 ± 0.14* 0.1320**	0.64 ± 0.03 0.0114	4.14 ± 0.17 0.0737	2.02 ± 0.08 0.0360	0.62 ± 0.02 0.0110	25,077
	B (6) 8 h/6 mo	7.2 ± 0.09	38.2 ± 0.23	6.40 ± 0.10 0.1145	0.52 ± 0.03 0.0096	2.40 ± 0.06 0.0428	2.22 ± 0.05 0.0395	1.26 ± 0.04 0.0224	31,022
II	A (5) 16 h/2 mo	3.4 ± 0.10	34.7 ± 0.26	5.67 ± 0.08 0.1010	0.56 ± 0.04 0.0101	1.74 ± 0.04 0.0310	1.75 ± 0.04 0.0311	1.60 ± 0.04 0.028	25,852
	B (4) 16 h/4 mo	2.6 ± 0.11	25.9 ± 0.19	6.90 ± 0.13 0.1230	0.64 ± 0.08 0.0114	1.49 ± 0.08 0.0265	2.35 ± 0.06 0.0418	2.42 ± 0.07 0.0431	21,187
	C (3) 16 h/6 mo	1.4 ± 0.08	10.4 ± 0.30	5.97 ± 0.11 0.1063	0.47 ± 0.05 0.0084	1.47 ± 0.06 0.0262	1.91 ± 0.06 0.0340	2.12 ± 0.06 0.0378	17,198

A/B, amacrine/bipolar synapse; A/A, amacrine/amacrine synapse; A/D, amacrine/dendritic synapse. Numbers in brackets indicate number of animals in each group.

exhibited well preserved receptors and were therefore taken as a control group. The outer retinal layers, formed mainly by the receptors, become thinner as the receptors degenerate. This decrease in the thickness is correlated with an increase in the daily illumination and with an increase in the period of exposure (Table). In general, changes in the outer plexiform layer (OPL) occurred earlier and were more severe than in the outer nuclear layer (ONL). The receptor terminals located in the OPL were damaged before and to a greater extent by illumination then other parts of the receptors. This agrees with the observation that receptor terminals shrink with light exposure as brief as 3 min in rats which had previously been kept in the dark[9]. The dimensions of the inner retinal layers did not vary significantly under the different light regimens and have not been included in the table.

Electron microscopy. The receptors are connected with bipolar cells which relay information from them into the inner plexiform layer (IPL). This is a layer of neuropil where processes of 3 elements, bipolar, amacrine and ganglion cells contact each other in conventional contacts which have on the presynaptic side an amacrine ending (amacrine/bipolar, amacrine/dendritic and amacrine/amacrine) and in a complex contact – a dyad – with a bipolar terminal on its presynaptic side and 2 dendrites or amacrine endings or one of each on its postsynaptic side[10, 11].

Electron micrographs of the IPL of retinae with damaged receptors showed, in agreement with the observation of O'STEEN et al.[5], little effect at the magnification used (26,600 ×). However, the big bipolar terminals, next to the ganglion cell layer, were considerably diminished in size or absent. Occasionally altered profiles of dendrites (which were either dark or swollen) and of amacrine end-

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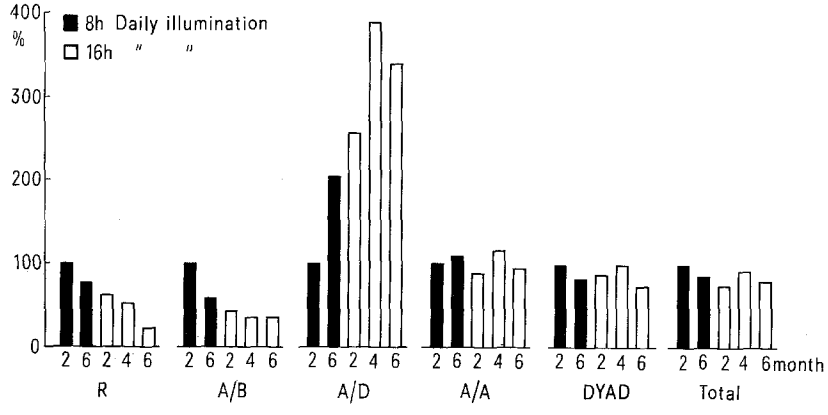

Fig. 2. Density of different synaptic contacts in the IPL (A/B, amacrine/bipolar; A/D, amacrine/dendritic; A/A, amacrine/amacrine and dyads) and the thickness of the receptor layers (R) under various light conditions expressed in percentage of the control group illuminated daily 8 h for 2 months.

ings (which were swollen and contained vacuoles) were observed. Both elements, however, retained their synaptic contacts (Figure 1).

The organization of the IPL relative to receptors suggested a different reaction to prolonged illumination for the various types of synapses. Therefore the synaptic density for each type of contact was determined (Table). Since after 8 h daily illumination for 2 months (group IA) the visual receptors were well preserved, the synaptic densities in the IPL of this group were taken as the reference value and data of all other groups were expressed as a percentage of this value (Figure 2). The density of the amacrine/amacrine contacts was only slightly affected by the length of light exposure, and a mild decrease in the density of dyads occurred. However, the density of amacrine/bipolar synapses was considerably decreased after prolonged illumination, and that of amacrine/dendritic contacts was significantly increased (Figure 2).

Discussion. Since bipolar elements are directly connected with visual receptors they can be expected to reflect the degree of damage to the receptors. This transsynaptic effect manifests itself by a loss of contacts in which the bipolar endings participate either pre- or postsynaptically (amacrine/bipolars and dyads). Similarly, severing the optic nerve induced transsynaptically a decrease in the synaptic density of the visual cortex[12].

Amacrine cells are not directly connected with the receptors, so that the loss of the latter does not affect contacts involving exclusively amacrine endings (amacrine/amacrine synapses). However, the amacrine endings become, by the loss of bipolar endings, deprived of one of the main sites of termination. They seem to make new contacts instead on dendrites and the formation of amacrine/dendritic synapses is inversely related to the density of bipolar synapses. Such a translocation would require formation of new dendritic surface to accomodate the amacrine endings. Since bipolar terminals contact, besides the amacrine endings also the ganglion cells and their dendrites, both amacrine and ganglion cells become partly denervated after the degeneration of the bipolar terminals. Denervated tissue has been found to be a strong stimulus for growth and can cause either axonal sprouting or a change in dendritic morphology[13].

Two systems in the rat brain can serve as examples of the first mechanism, axonal sprouting. The septal nuclei receive 2 main inputs: from the hippocampus and from the hypothalamus. A long-term lesion in one system induces the spread of terminals of the other system to sites originally occupied by the first, and vice versa[14]. Similar synaptic rearrangement occurs in the superior colliculus after long-term unilateral enucleation[15]. Altered dendrites of anterior horn cells observed proximal to the site of hemitranssection of the spinal cord could serve as an example of the second mechanism[16]. Varicosities developing on the dendritic tree increase the potential synaptic sites. Since the amacrine and ganglion cells become partially denervated structures, either of the above mechanisms could then trigger a process resulting in an increased number of amacrine/dendritic contacts.

Zusammenfassung. Nachweis, dass die Netzhautrezeptoren der weissen Ratte durch Lichtexposition beschädigt werden. 16 h Beleuchtung täglich verursacht nach 6 Monaten eine drastische Rezeptorenabnahme, wodurch die synaptische Organisation der inneren plexiformen Schicht geändert und die Dichte der Synapsen vermindert wird.

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On the Presence of Glycogen in the Rhombencephalon of Chick Embryos During Development

Although the distribution of glycogen in the nervous system of adult and embryonic animals has been investigated in many vertebrate species, few studies on its distribution in birds are available. GAGE[1], using the iodine reaction and salivary digestion, found that glycogen appears late in the development of the nervous system in chick embryos with respect to Petromyzon and Amblystoma.

Glycogen is abundant in the medulla oblongata and the lumbosacral region of the spinal cord from the 6th to the 10th day of development, although its precise location has not been reported. GAGE states that the production and utilization of glycogen is a property that the protoplasm of nervous cells shares with the protoplasm of any other cell. Glycogen is essential to nervous tissue, as it is to all other tissues during histogenesis, especially in the transition to their definitive and functional state. Glycogen is an energy reserve, and its appearance in all vertebrate tissues depends, at least in part, on the time at which the tissue becomes functional. It therefore appears early in the development of Amblystoma and later in the chick embryo.

In the nervous system of higher vertebrates, Gage did not find demonstrable quantities of glycogen after the embryonic period, since after this stage liver and muscle assume the principal glycogenetic functions.

The accumulation of glycogen in nervous tissue during the proliferative process, and its disappearance during histological differentiation as reported by GAGE[1] in the chick embryo and by JANOSKY and WENGER[2] in Amblystoma, has not been confirmed by SACCANI and MARINI[3] in urodele amphibians.

MASAI[4] found seasonal variations of glycogen content in the nervous system in amphibians, fish and reptiles. In

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