EFFECTS OF LIGHT DEPRIVATION AND LIGHT DAMAGE ON THE ULTRASTRUCTURE OF THE INNER PLEXIFORM LAYER OF RAT RETINA

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by

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Abstract

Four groups of eight male albino rat pups (14 days old) were raised for 8 weeks under different conditions; 1) unsutured, bright light reared (UB), 2) bilaterally lid-sutured, bright light reared (SB), 3) unsutured, dim light reared (UD), and 4) bilaterally lid-sutured, dark reared (SN). The intensity of the bright light was 1960 lux. The intensity of the dim light was equated with that striking the corneas of the SB group (transmission of the sutured rat eye lid is about 0.5%), and was therefore 9.8 lux. All retinas were fixed in osmium, followed by glutaraldehyde and sectioned and stained for light and electron microscopy. Microtomy, microscopy, and scoring were done blind.

Light microscopy showed that the retinas of both the UB and SB groups were extensively damaged--almost complete degeneration of the outer retinal layers, indicating that even very low intensity light (9.8 lux), when continuous, causes severe retinal damage. The SN group was thicker in many of the retinal layers compared to the UD group.

Electron microscopy revealed that, with the exception of amacrine-ganglion synapses, there are no significant changes in the incidences of any type of synapse in the IPL of the rat retina after light deprivation or light damage. The incidence of amacrine-ganglion synapses was significantly greater ($p < .05$) in groups UB and SN, only in the outer third of the IPL.

In light of the present results, plasticity in the rat retina is questionable. If it does exist, it is a very small effect, and is probably reflected by only the amacrine-ganglion synapses.

Introduction

Retinal Anatomy.

Early anatomical studies have shown that all vertebrate retinas are qualitatively similar (Cajal, 1972; Polyak, 1941; Boycott & Dowling, 1969; Dubin, 1970). The retina is composed of 5 different types of cells, with their nuclei arranged in 3 nuclear layers, separated by two layers of synaptic interaction that are free of nuclei (plexiform layers). The receptor perikarya form the outer nuclear layer (ONL) of the retina, which is separated from the inner nuclear layer (INL) by the outer plexiform layer (OPL). The INL contains the cell bodies of the horizontal, bipolar, and amacrine cells. The horizontal cell nuclei are usually found to border the distal edge of the INL, and the amacrine cell nuclei found to border the proximal edge, while the bipolar cell nuclei are seen mainly in the middle of the INL. The inner plexiform layer (IPL) separates the ganglion cell layer (GCL) from the INL. The GCL is composed of the ganglion cell bodies, whose axons form the optic nerve fiber layer. The plexiform layers contain the fine processes of the cells from adjacent nuclear layers and are the sites of their synaptic interaction. Communication between receptor, horizontal, and bipolar cells occurs in the OPL, and communication between bipolar, amacrine, and ganglion cells occurs in the IPL. Bipolar cell axons have a specialized presynaptic ribbon, which is usually directed between two postsynaptic processes, one of which is amacrine and the other either a ganglion cell dendrite or another amacrine process. This type of synapse has been termed a dyad (Dowling & Boycott, 1966). The amacrine cells form conventional synapses, and may have either bipolar, amacrine, or ganglion cell processes as

postsynaptic elements. The ganglion cell dendrites are never presynaptic.

Of the two layers of synaptic interaction, the IPL has the more complex structure (Cajal, 1972; Dowling & Boycott, 1966), and has recently been shown to have a correspondingly greater role in establishing complexity of retinal output (Werblin & Dowling, 1969). Dubin(1970) has shown that animals ranked according to anatomical complexity of IPL are similarily ranked according to complexity of the response properties of ganglion cells, i.e. human, monkey, cat, rat, rabbit, ground squirrel, frog, and pigeon. An indication of the degree of anatomical complexity is the ratio of amacrine synapses to bipolar synapses. The amacrine:bipolar (A:B) ratio is the quotient of the incidence of amacrine (or conventional) synapses and the incidence of bipolar (or ribbon) synapses per unit area. Table 1 shows the A:B ratios obtained from "normal" or control retinas of the main studies on rat IPL. Large differences in the ratios are apparent and it will be argued later that they are likely due to differences in methodology and criteria for identification of synapses.

Physiology of the Retina:

The anatomical and physiological studies of the retina indicate that it is a good model with which to study neural function as it has only one input and one output, yet it is complex enough to be interesting. Investigators have found correlations between the physiology and the anatomy of retina. Dowling and Werblin (Dowling & Werblin, 1969; Werblin & Dowling, 1969) have found that the central part of a bipolar cell receptive field closely approximates its dendritic spread, and similarily, the lateral spread of the horizontal cell processes approximate the antagonistic surround of the bipolar cells. It is thought that the antagonistic

surround is formed by receptor driven horizontal cells in the periphery of the bipolar's receptive field which modulate the bipolar output (Werblin & Dowling, 1969). The amacrine cell is considered an interneuron in the IPL and probably serves to establish strong antagonistic center surround properties in ganglion cells. Amacrine cells, because of their transient nature, are also thought to be responsible for the phasic, motion sensitive ganglion cells (Werblin & Dowling, 1969). Werblin & Dowling(1969) suggest that ganglion cells which are tonic in nature receive mainly bipolar input, and those which are phasic in nature receive mainly amacrine input. The motion-selectivity of ganglion cells is also thought to be the result of amacrine cell mediation (Dowling, 1970). Thus the known physiology of retinal components strongly implies its relationship to retinal anatomy.

Plasticity of Rat Retina.

The ease with which natural stimuli can be presented and controlled, and the fact that it has one output which can be easily monitored, make it very advantageous to use as a model tissue for studying neuronal integration. The retina would, thus, seem ideally suited for investigations of anatomical plasticity of the nervous system. Sosula & Glow (1970, 1971) and Fifkova(1972b) have thoroughly described the synaptic densities in the IPL of the normal rat retina and have in addition demonstrated a surprising plasticity in this structure which consists of changes in the incidences of synapses in this layer. These investigators agree that after visual deprivation by monocular lid-suture (Fifkova, 1972b) or occluder (Sosula & Glow, 1971), there is a significant increase in the total number of amacrine synapses in the IPL. However, beyond this, there is substantial disagreement between these two investigators. Table 2 compares

Table 1. The incidences and A:B ratios of "normal" or control retinas from the main studies in the literature.

	Amacrine Incidence/μ^2	Bipolar Incidence/μ^2	A:B
Dubin (1970)	0.0725	0.0220	3.3
Sosula & Glow (1970)	0.0560	0.0080	7.0
Sosula & Glow (1971)	0.0620	0.0081	7.7
Fifkova (1972b)	0.1207	0.0114	10.6

Table 2. Comparison of the increases of amacrine synapses in a light deprived retina to a control retina in studies by Sosula & Glow (1971) and Fifkova (1972b). A/(total) = total amacrine synapses, A/A = amacrine-amacrine synapses, A/B = amacrine-bipolar synapses, A/G = amacrine-ganglion synapses.

Synapse	Sosula & Glow (1971)		Fifkova (1972b)	
A/(total)	2.4 X increase	.001	1.5 X increase	
A/A	2.2 X increase	.001	1.7 X increase	.001
A/B	1.5 X increase	ns	1.2 X increase	.001
A/G	3.5 X increase	.001	0.9 X decrease	ns

the increase of amacrine synapses due to light deprivation, and the areas of disagreement between the two studies are evident. Sosula & Glow(1971) found a 1.5 fold increase in the number of amacrine-bipolar synapses, and Fifkova(1972b) found a 1.2 fold increase, and although the direction of change was similar for both studies, they disagree as to statistical significance. Sosula & Glow(1971) found a 3.5 increase in the number of amacrine-ganglion synapses, whereas Fifkova(1972b) found a nonsignificant 0.9 fold decrease.

Some recent studies have demonstrated retinal degeneration due to what was previously thought "low level" illumination (Bennett, et al., 1973a, 1973b). This suggests that retinal plasticity may be caused by light damage rather than light deprivation. Fifkova(1972b) suggests that differences between her and Sosula & Glow's(1971) data may be due to this additional variable as Sosula & Glow(1971) used hooded rats which might have been more resistant to light damage than her albino rats.

The effects of light damage are most readily observed in the outer layers of the retina (the outer plexiform and photoreceptor layers). Degeneration of the outer layers has been observed after a period of continuous light exposure (Anderson & O'Steen, 1972; Gorn & Kuwabara, 1967; Noell, et al., 1966; Noell, et al., 1971; Noell & Albrecht, 1971; O'Steen & Anderson, 1972), and more specifically, extensive loss of receptor cells after 120 days of continuous "normal light" (approximately 400 lux) was observed by Bennett, et al. (1973b). Photoreceptor and pigment epithelial cell damage after 6 and 12 hours of light often considered normal (70 foot candles direct light, and 18 foot candles reflected from cage bottom; 754 and 194 lux respectively) was found to be reversible after 4 hours of dark (Shear, et al., 1973), but it is uncertain whether 18

hour damage is reversible. Fifkova(1972a) observed that shrinkage of the outer layers was dependent on the duration of light exposure. In addition, Lawwill(1973) has confirmed that, in rabbit, not only is the threshold of permanent retinal damage dependent on the light source, but the damage is directly related to the absorption of energy by the receptors, and that light of the visible spectrum is responsible for the damage.

Although most studies concerning light damage have observed the effects on the outer layers, changes within the IPL are also thought to occur. Weiskrantz(1958) has observed a decrease in the thickness of the IPL in light deprived cats, which has been confirmed by Rasch, et al. (1961), but Cragg(1969) suggests that these findings are in opposition to these authors, but his main concern was OPL, not IPL. The findings by others (Sosula & Glow, 1971; Fifkova, 1972b) suggest that there is no significant change in thickness of IPL due to light deprivation, or light damage (Fifkova, 1973).

Fifkova(1972b) observed a reduction in the number of bipolar synapses in the IPL due to "normal" light (500 lux, 8 hours per day for 2 months), and also observed there was no difference in the thicknesses of the outer layers of the retinas of both the deprived and undeprived animals. Sosula & Glow(1971) also found no decrease in the thickness of the outer layers in the exposed retinas of hooded rats, but did not confirm a significant decrease in the number of bipolar synapses and in fact found the direction of change to be opposite (See Appendix F). Fifkova(1972b) suggested that this difference might be due to protection offered by the pigment in the choroidea of hooded rats.

Fifkova(1973) has further investigated light damage in the IPL. She raised rat pups (14 days old) with 500 lux under the following time schedule: 8 hours per day for 2 months, 8 hours for 6 months, 16 hours for 2

months, 16 hours for 4 months, and 16 hours for 6 months. As in her earlier study (Fifkova, 1972a) she found no evidence of damage to the receptor layers in the group exposed to 500 lux, 8 hours per day for 2 months, and so this group was considered a control. It was found that increasing the length or period of exposure resulted in increased receptor layer damage, and also a decrease in the amacrine-bipolar incidence and a significant increase in the amacrine-ganglion incidence. Her groups are in effect many replications of the light damage effect, and the consistency of the results make it seem likely to be a real effect.

The discrepancies between Sosula & Glow's(1971) and Fifkova's (1972b) studies are difficult to resolve, partially due to methodoligical differences. Sosula & Glow(1971) used adult hooded rats (3 months old), whereas Fifkova(1972b) used albino rat pups (lid-sutured at 14 days). Fifkova (1972b) suggests that the natural pigment of the choridea of the hooded rats may act as protection from light damage. Sosula & Glow(1971) used the open eye of their monocularly occluded rats as a control, and so may have been making a different comparison than Fifkova(1972b), who used undeprived, unsutured animals as controls. Sosula & Glow(1971) may have compared light deprived retinas with light damaged retinas if they used the open eye of the monocularly deprived rats as the controls, although this is less certain in the case of hooded rats (Fifkova, 1972a). The fixation techniques were very dissimilar. Sosula & Glow(1971) immersed whole retinal cups in osmium. Tissue fixed in osmium alone yields low-density micrographs, making identification of the usually higher density bipolar cells difficult. Fifkova's(1972b) fixation makes identification of different cell processes more certain as the bipolar terminals have a matrix with an overall higher density than the amacrine and ganglion

cell dendrites. In fact, Sosula & Glow(1971) used the presence of a ribbon synapse as the criterion for a bipolar cell, rather than the entire bipolar cell matrix. Sosula & Glow(1971) stained their thin sections with uranyl acetate, followed by lead citrate, as opposed to Fifkova(1972b) who stained with lead citrate only. Yazulla(1974) has shown that double staining should be preferred, as this method tends to stain smaller ribbons, left unstained by lead citrate alone.

Sosula & Glow(1971) compared one montage from a deprived retina with one montage from their control retina, along with sets of 5 isolated micrographs which bias for bipolars. Fifkova(1972b) compared micrographs taken randomly throughout the IPL, rather than montages, of 7 deprived retinas with those of 3 controls. When comparing only one deprived retina with one control retina, and using random micrographs rather than full montages, it would certainly be easy to find significant differences. Results from electron microscopy are at times very difficult to interpret when using small sample sizes. Consistent results are difficult to obtain, thus significant differences between only 2 samples may be the result of artifact. Unless all retinas are fixed at the same time, slight differences in fixation procedure, or different batches of fixative may influence the results. The magnification properties of an electron microscope may change during the project (e.g. if the scope is repaired for some reason, and the lens current adjusted, or even just drift over time) to significantly change the incidence counts. Also, changes in lens strength even during one session can vary magnification easily by $\pm$ 5%, which gives an area range of 20%, therefore use of a calibration grid is a must.

If the samples were taken from different areas of retina, the

incidence counts may be significantly different due to systematic variations of synaptic densities between the center and periphery of retinas in general. It is imperative that similar areas of retina are compared. Fifkova(1972b) observed samples from a "strip of tissue 1 mm wide, from which blocks next to the optic nerve were prepared". Sosula & Glow(1971) do not indicate how, or if, they were certain of looking at similar areas in their comparison of light deprived and control retinas. It is difficult to resolve whether the discrepancies between the two studies are due to their methodological differences, methodological problems, or both.

The IPL changes may be due to changes in synaptic size rather than incidence. Some evidence exists which suggests that the decrease in the total bipolar incidence is due to a size difference rather than a number difference. Wagner(1973) has shown a dark-induced decrease in the number of synaptic ribbons in fish retina, and suggests that the "restitution" of the same ribbons to allow photopic vision must take place in less than 30 minutes. This rapid change in ribbons brings up the possibility that the differences in bipolar incidences due to light deprivation may be relative size differences rather than absolute differences. Bipolar synapses may not be decreasing in number, but decreasing in size, accounting for the decreased bipolar incidences observed by Fifkova(1972b). (As noted before, Sosula & Glow(1971) find this effect nonsignificant, and even have results in the opposite direction!)

Some recent evidence by Sherman & Stone(1973) suggests that visual deprivation by lid-suture has no effect on the development of the retina, at least in cat. In an attempt to locate the site of the physiological abnormalities in light deprived cats, field potentials and single unit characteristics of Y, X, and W type ganglion cells of light deprived

cats were compared with normal cats. Both the antidromic field potentials and the single unit characteristics were normal. The integrity of the ganglion cell characteristics depends very much on that of amacrine and bipolar input. It is reasonable to assume that if there was a large increase in amacrine input onto ganglion cells, that their response characteristics would change, perhaps making them more phasic (recall Werblin & Dowling, 1969), but no such change is observed.

The present literature on the IPL of rat retina and on the effects of visual deprivation are very inconsistent. The comparison in table 1 of normal or control retinas show Dubin(1970) to have found an A:B ratio of 3.3, Sosula & Glow(1970) 7.0, Sosula & Glow(1971) 7.7, while Fifkova (1972b) had 10.6. The discrepancy for normal retinas is very large and further investigations dealing with light deprivation are also inconsistent. As seen from table 2 and Appendix F, Sosula & Glow(1971) and Fifkova(1972b) are statistically different, and even in opposition concerning the changes in the IPL. Only one investigator has looked at the effect of light damage on rat retina (Fifkova, 1973). Those studies on light deprivation have manipulated both patterned light and diffuse light simultaneously, and have not adequately compared the effect of light damage. One study is needed to investigate the individual effects of form deprivation, light deprivation, and light damage on the ultrastructure of the IPL of rat retina to resolve the differences, be they due to the methodological differences or methodological problems of the present literature.

If environmental differences impose plasticity at as early a level as the retina, it becomes debatable whether the monocularly induced plasticity observed at the visual cortex indeed originates in cortex alone, or actually is due to an altered input as a result of changes within

the retina. (However, binocularly induced plasticity is certainly cortical in origin as this is the first place that input from both eyes interact). If plasticity is indeed found in a structure as early as retina, it must give pause for thought to investigators who claim that the cortical plasticity they have observed is the basis of memory. Thus it is important to resolve the inconsistencies in the literature by testing plasticity more critically and, if verified, to determine which environmental factors are responsible for specific retinal changes.

The present study was an investigation intended to resolve the effects of form deprivation, light deprivation, and light damage on the IPL of rat retina. To clear up the inconsistencies of the previous studies, litter-mate rat pups were used, and run at the same time. All animals were fixed at the same time, and the microtomy, microscopy, and scoring were done blind. To ensure true magnification (and incidence counts), a calibration grid was used with each montage and light micrographs. A strict criterion (Dubin, 1970) was adhered to and checked by an independent observer for uniformity of scoring.

Results for the light deprived retinas and light damaged retinas were opposed to those found by Sosula & Glow(1971) and Fifkova(1972b, 1973), in that there were no significant differences between synaptic incidences in the IPL of light deprived and light damaged retinas. There were in fact no significant differences between the IPL of retinas exposed to bright light or dim light, or those light deprived. The results obtained by Fifkova(1972b) and Sosula & Glow(1971) will be discussed in the light of the present findings.

Methods & Materials

Three male albino rat pups from each of eight triplet sets were randomly assigned to one of three conditions: 1) unsutured, bright light reared (UB), 2) bilaterally lid-sutured, bright light reared (SB), and 3) unsutured, dim light reared (UD). A fourth group, bilaterally lid-sutured, dark reared (SN), was composed of eight male albino rat pups randomly chosen from six litters. The experiment originally proposed to use eight littermate quadruplet sets, with each member of a set assigned to a condition, such that a litter effect, if any, could be controlled. In the course of the experiment, the female with the SN group became vicious, and cannibalized the entire group. This group was replaced with pups chosen randomly from six litters born on the same day. All pups were placed in their respective conditions on day 14, along with one nursing female per group. The females were removed after 6 days, and the pups remained in their conditions for a total of 8 weeks.

To accomplish lid-suture, the edges of the upper and lower lids were surgically removed, under ether anesthetic, and the lids were then sutured together (Ethicon K-889, 6-0 sterile surgical silk) and allowed to heal. This method ensures that there are no minute holes for direct light to enter. All suturing was done on day 14, before the eyes had opened.

The four groups of pups were housed in identical 50 cm X 45 cm X 12.5 cm wire mesh cages (see figure 1). Groups SB and UB were exposed to the same bright light source. The intensity of the bright light was 1960 lux, and was provided by a series of 20 inch, 20 watt flourescent tubes. To ensure that the light was uniform, a sheet of white cotton material served to diffuse the light. The UD group was exposed to low

Figure 1. Example of one of the 50 X 45 X 12.5 cm wire mesh cages used to house the rat pups. Each cage housed 8 rat pups from day 14 for a period of 8 weeks.

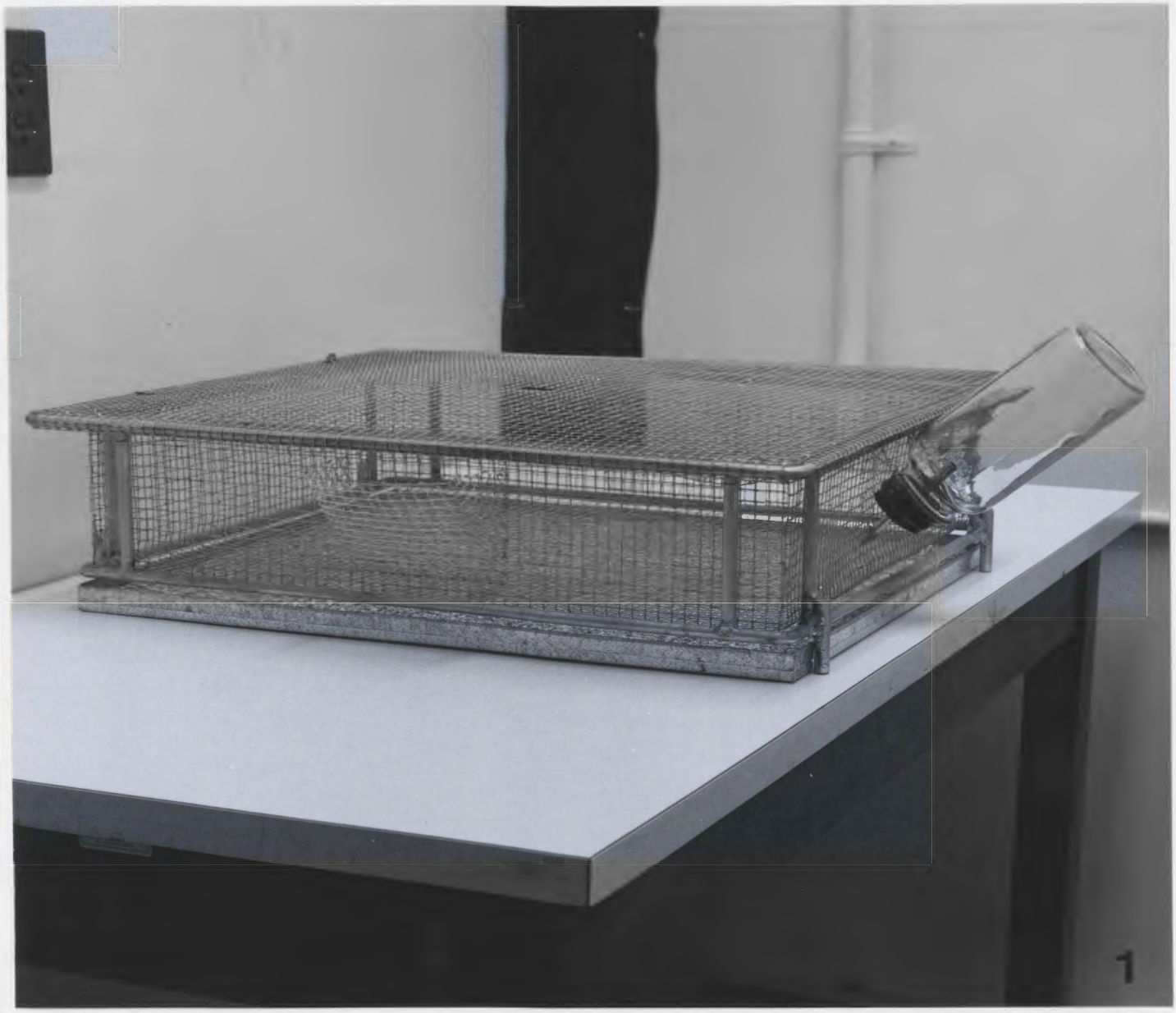
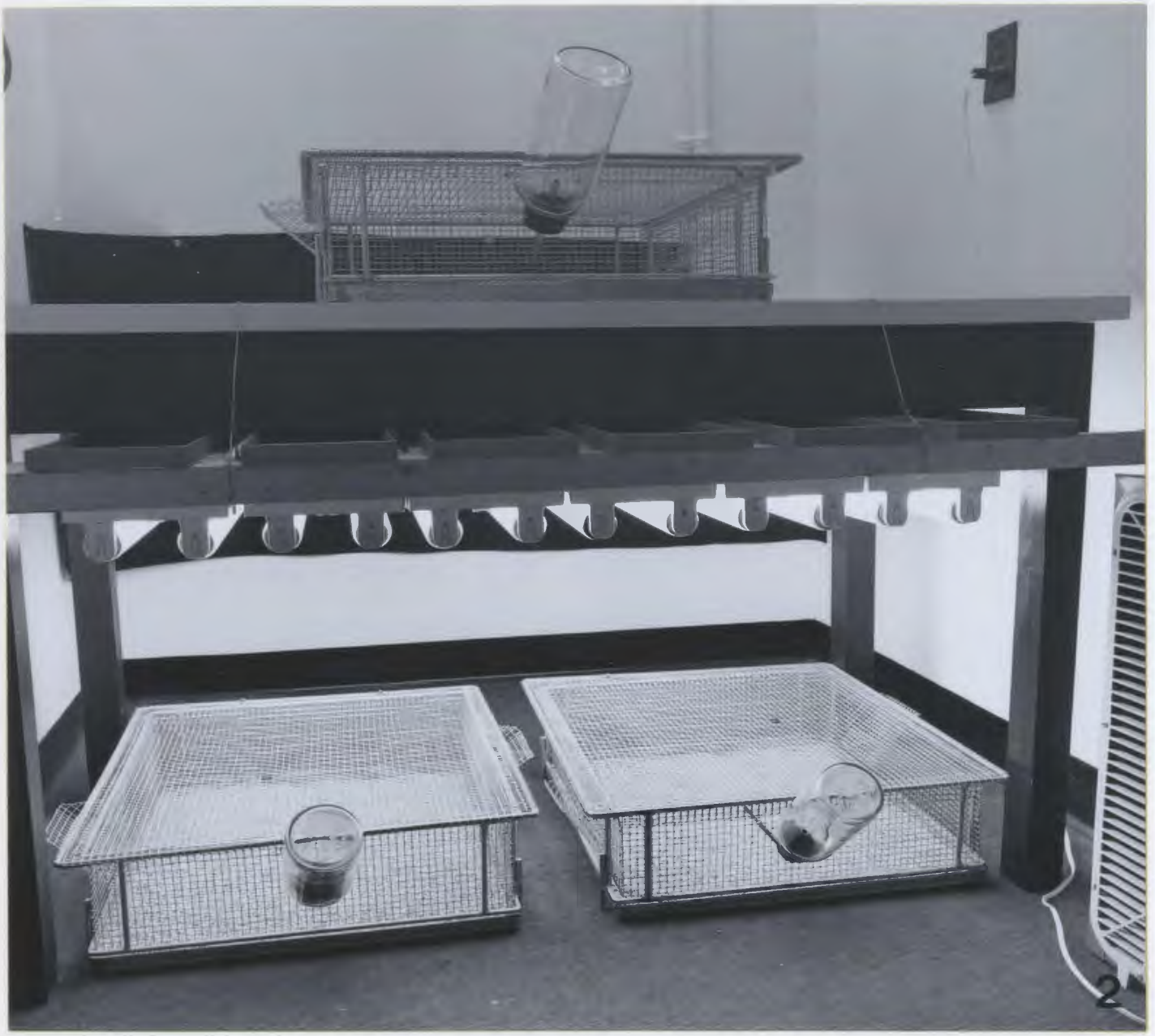

1

Figure 2. View of the experimental set-up without the diffusing sheets in place. The two cages below the battery of lights housed the unsutured, bright light, and the lid-sutured, bright light groups, and the cage on top of the table housed the unsutured, dim light group. The fluorescent tubes were each 20 watts and 20 inches long.

Figure 3. View of the experimental set-up with the diffusing sheets in place. The diffusing sheets adjusted the light intensity of the bright light groups to 1960 lux, and that of the dim light group to 9.8 lux.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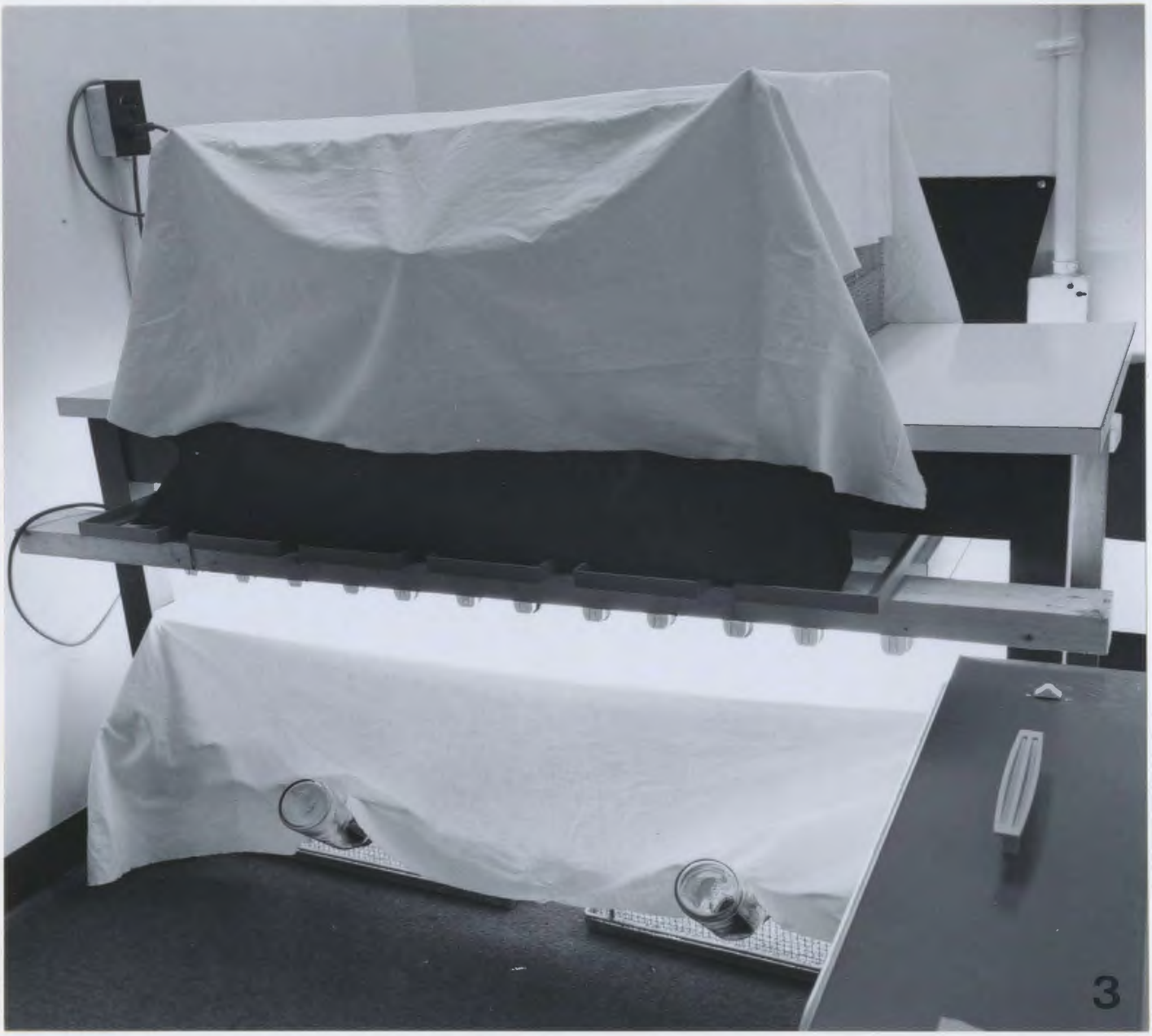
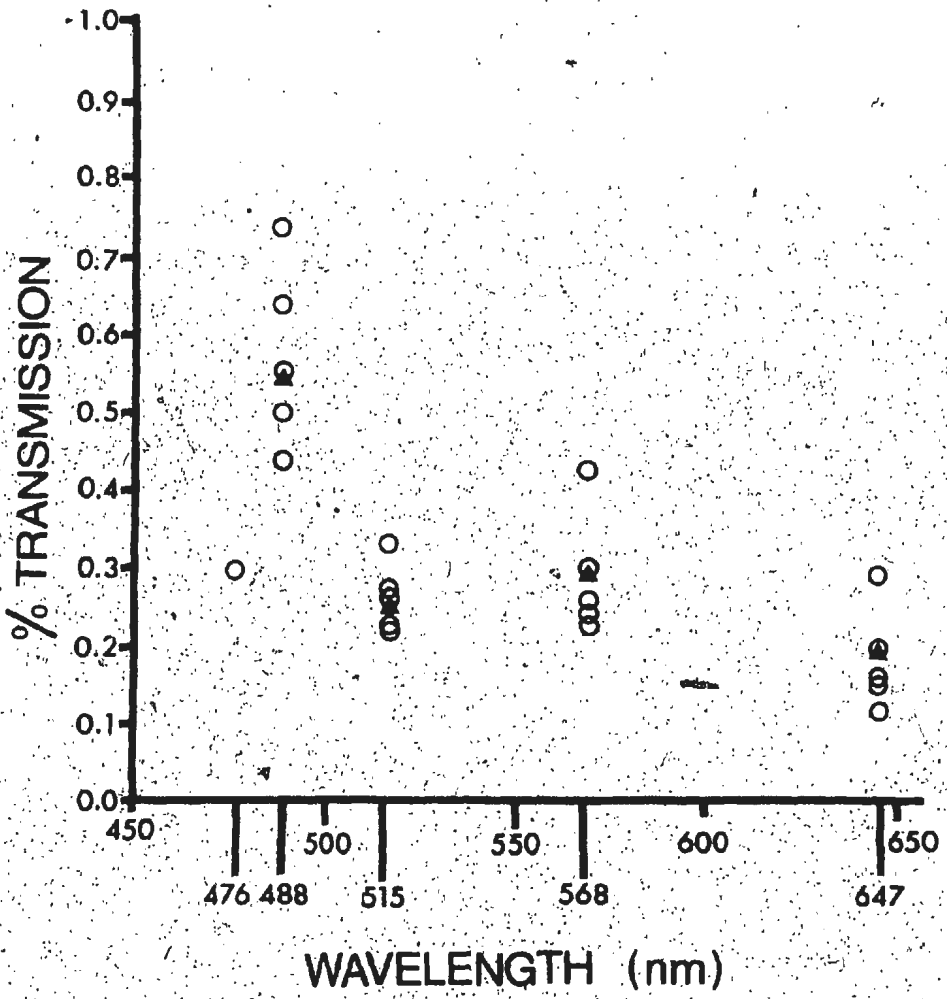

3

intensity light. The light source for this group was provided by the flourescent ceiling lights of the room. A sheet of white cotton material was used to adjust the intensity to about 9.8 lux, and also served to diffuse the light. Figure 2 shows the experimental set-up without the diffusing sheets and figure 3 with the diffusing sheets. All measurements of intensity were taken at the cage bottoms, with a Gollen 8.66-1072 light meter. The exact intensity of the diffuse light for the UD group was matched with the calculated intensity of light incident on the corneas of the SB group. (The intensity of light striking the corneas of the SB group is the product of the intensity of the light at the cage level and the proportion of light transmitted by the eye lid.)

The percent transmission of the sutured rat eye lid was measured in the following manner. Eye lids of one month old rats, previously lid-sutured at day 14, were trimmed of hair, surgically removed, mounted across an aperture 7 mm in diameter, and immediately placed in the path of a laser beam produced by a Model 52 Ion Laser (Coherent Radiation, Palo Alto, Calif.). The incident and transmitted light was detected by a photocell and measured on a calibrated meter. Transmission coefficients were 0.5% to 0.1% for wavelengths ranging from 488 nm to 647 nm respectively (see figure 4). Similar results were obtained using a Unicam SP 500 Series 2 Ultraviolet and Visible Spectrophotometer (0.5% or less transmission), but the light source was not intense enough for a more accurate measure. Thus, the lid-sutured bright light reared rats had to receive about 200 times as much light (1960 vs. 9.8 lux) as the unsutured, dim light reared rats in order to equate the luminous flux reaching their retinas.

Figure 4. Graph of the per cent transmission of the sutured rat eye lids for the given wavelengths. The circles (O) represent the per cent transmission of rat eye lids for the indicated wavelengths from a laser source. The triangles (▲) represent the mean per cent transmission for a particular wavelength. The high transmission at 488 nm is thought to be due to a very narrow "window" near the blue area of the spectrum. The peak would then be nearer the red end (toward 647 nm) of the spectrum.


% TRANSMISSION
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
450
500
550
600
650
476
488
515
568
647
WAVELENGTH (nm)

propylene oxide-Epon, at which time they were placed in 100% Epon in rubber moulds and left in a 50° C. oven for 2 days. See Appendix A for a detailed description of solutions for fixation, and also a working schedule. Pilot work has shown that in rat retina, osmium alone gives the best fixation of membranes, but results in extraction of much of the cytoplasmic matrix, making identification of some cell profiles difficult. Glutaraldehyde followed by osmium yields good contrast micrographs in which cell types are easily identified by differences in their matrices, but tends to explode the mitochondria and results in poor membrane fixation in general (possibly making it harder to identify small synapses). It was found that the osmium followed by glutaraldehyde fixation tended to preserve the membranes very nicely, as well as maintaining some of the cytoplasmic matrix. Thus this fixation was used as a compromise between observability of synapses and identifiability of processes.

A total of 20 retinas (5 from each condition) were selected on the basis of good fixation and ability to localize the chosen area. To ensure that all subsequent work was done blind, the retinas were randomly coded within littermate blocks by a naive observer. The microtomy, microscopy, and scoring were then done one randomized block at a time in order to minimize any progressive bias. All sectioning was done on an LKB Ultramicrotome. One micron sections were cut and stained with 1.0% paraphenylenediamine for light microscopy. Silver sections (Peachy, 1958) of IPL were cut and mounted on copper slot grids, and stained with 2% uranyl acetate (about 20 min.) followed by full strength Reynolds' lead citrate (Reynolds, 1963) (about 10 min.). Yazulla(1974) has found that double staining is preferred because it stains smaller ribbon synapses ordinarily weakly stained using lead citrate alone, and therefore often unnoticed.

All groups received food (Purina Rat Chow) and fresh water ad lib. To dissipate the heat produced by the battery of lights in the "light" room, the air was continuously circulated by an electric fan. The temperature of the "light" room was taken daily from the top of one of the bright light cages. The mean temperature for the 8 week period was 27.15 ± 1.81 S.D.° C. The lid-sutured, dark reared group was kept in another light-tight room, also equipped with a circulating fan to help keep the temperature constant and supply noise to equate with that of the "light" room. The mean temperature for the 8 week period was 26.60 ± 1.65 S.D.° C. (The temperatures were originally taken 3-4 times per 24 hours, but this was found unnecessary, as there was little fluctuation.)

After a period of 8 weeks under these conditions, all rats were sacrificed, the eyes enucleated, and the retinas fixed according to a modification of West & Dowling(1972). After enucleation, the eyes were hemisected, and the posterior cup immediately immersed in ice-cold 2% Veronal acetate buffered osmium, made from equal volumes of 4% osmium tetroxide and Veronal acetate buffer. The time from severing of the optic nerve (and retinal blood supply) to immersion of the retinal cup in osmium was no greater than 70 seconds. After 90 minutes, the osmium was decanted, and replaced with a cacodylate wash, left on ice for 5 minutes, and then post fixed for 60 minutes in ice-cold 2.5% glutaraldehyde. The tissue was then dehydrated in a graded series of ethanol baths (50% for 5 min., 70% for 10 min., 85% for 10 min., 95% for 10 min., 3 changes of 100% in 20 min, with the last change to Absolute) and finally propylene oxide (3 changes over 20 min.). The retinas were let stand for 60 minutes (swirling occasionally) in 50:50 propylene oxide-Epon, then overnight in 20:80

To ensure that sampling was from similar areas of retina in all animals, the prominent blood vessels on the scleral surface and the optic nerve were used as landmarks. Figure 5 shows the area from which all samples were taken from all retinas. The orientation of the prominent blood vessels with respect to the optic nerve and the dorso-ventral axis was constant, and the temporal notch was cut during hemisection of the eye. The sampling area (stipled area) was approximately 1 mm temporal to the optic nerve, on a line drawn between the optic nerve and the temporal notch.

For examination of retinal layer thicknesses, light micrographs of thick (1 μ) sections were taken on a Zeiss Universal microscope and printed at a magnification of 900X. Photomontages of the entire depth of IPL were made of the 20 retinas from electron micrographs taken by a JEOL 100B electron microscope at a magnification of 20,000X. The magnification of the negatives was approximately 7,000, and prints were made at about 2.6X the negatives. A calibration slide was used with the light micrographs to check the magnification of the prints. To ensure true magnification of electron micrographs, a calibration grid was shot along with each montage of IPL.

Each IPL montage was divided into 3 equal layers: inner, middle, and outer third, and the frequency of each type of synapse was tabulated for each layer. The criteria for identification of the types of terminals and synapses in rat retina have been described elsewhere (Dubin, 1970; Fifkova, 1972b; Sosula & Glow, 1970, 1971). Two criteria for bipolar synapses were used: 1) the dyad, as described by Dowling & Boycott(1966) (see figure 14 & 15) and 2) the free-floating ribbon (see figure 14 & 15), which is not in the usual dyad configuration but can definitely be identified

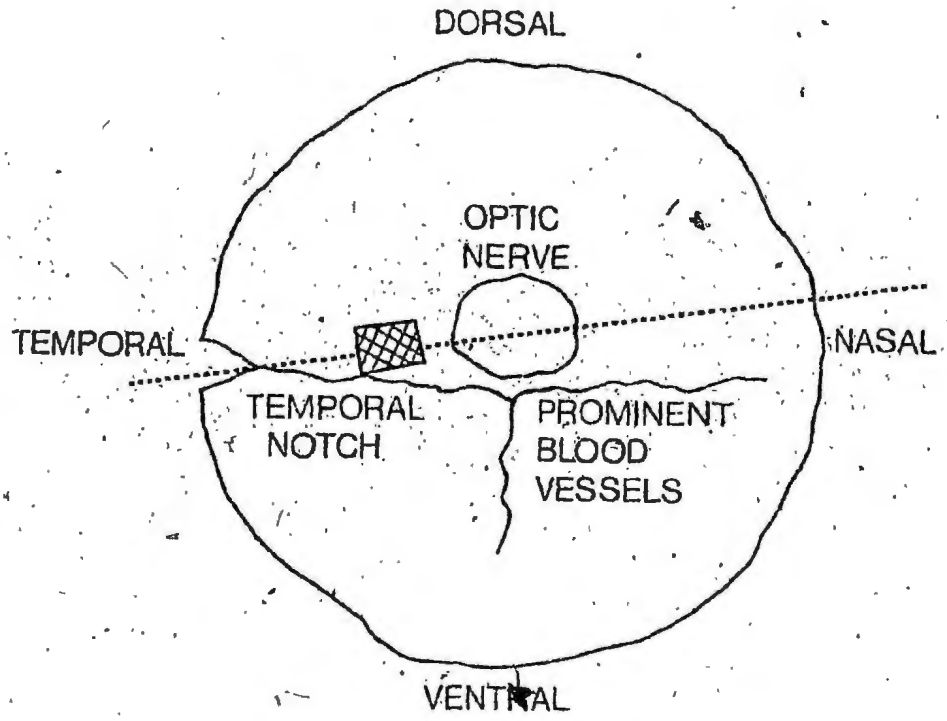

DORSAL
OPTIC NERVE
TEMPORAL
NASAL
TEMPORAL NOTCH
PROMINENT BLOOD VESSELS
VENTRAL

Figure 5. Schematic diagram of the scleral surface of a rat retina showing the landmarks used to locate identical areas (stipled) on all retinas examined. The area of observation is located 1 mm temporal to the optic nerve, on a line drawn from the center of the optic nerve to the temporal notch (cut during hemisection of the eye). See text for more detail.

as a ribbon. Dubin(1970) has found, that upon further sectioning, the free-floating ribbons are associated with a dyad synapse. Thus, free-floating ribbons were considered to be reliable indicators of bipolar synapses which were slightly out of the plane of section. For examples of scoring of synapses, see figures 12, 13, 14, and 15. Scoring of the photomontages was done one littermate set at a time, without knowledge of the history of the retina, and was continually checked by an independent observer.

Synaptic incidences were calculated by dividing the number of synapses by the area (in μ^2). These incidences along with the A:B ratios were statistically compared between the following groups: The effect of light deprivation was observed by comparing the SN and SB groups. Neither group received patterned input due to the lid-suturing, thus the only difference between the two groups was the presence or absence of diffuse light. Form deprivation was observed by comparing the SB and UD groups. Both groups received equated intensities of light, thus the only difference between these two groups was the presence or absence of form vision. Finally, comparison of the UD and UB groups shows the effect of light damage on the IPL.

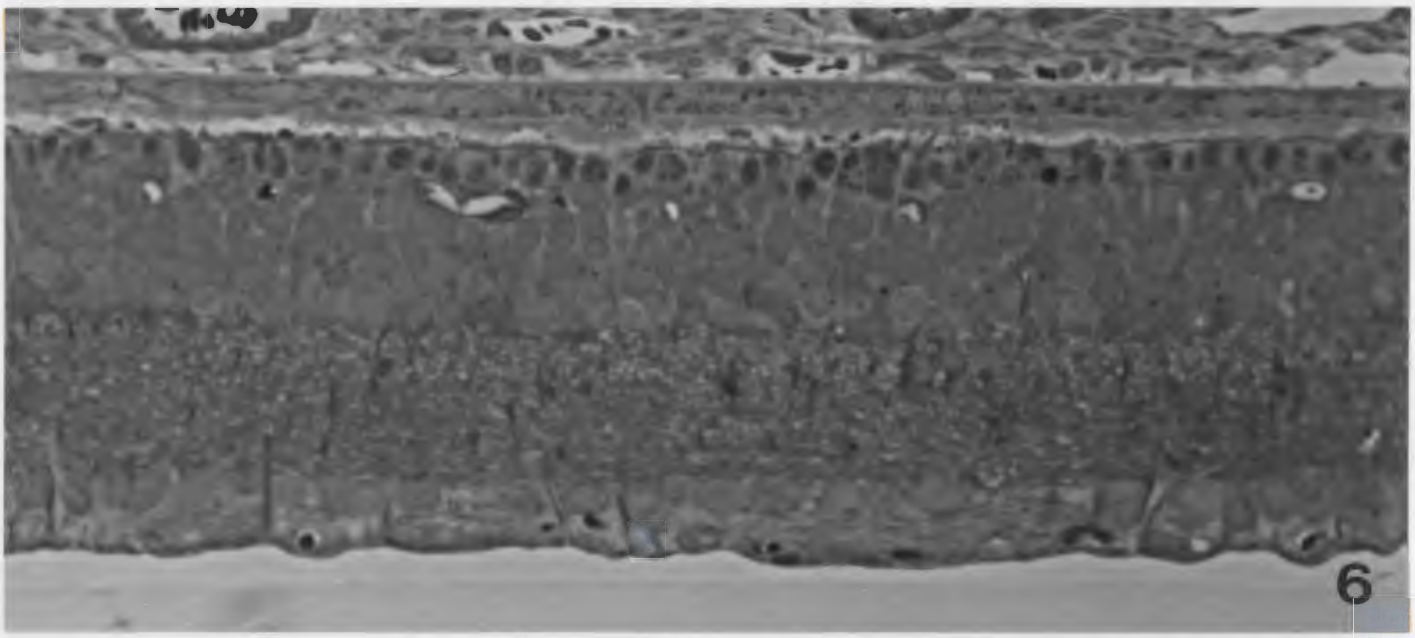
Results

Light microscopy.

Measurements of the thicknesses of retinal layers under the four rearing conditions are summarized in table 3. (Measurements of individual retinas within any group can be seen in Appendix B.) An analysis of variance, and, where necessary, a Newman-Keuls (see Appendix C), were done for each layer across rearing conditions. The mean lengths of the outer segments of both the unsutured, bright light and the lid-sutured, bright light groups was the same, and they were significantly smaller ($p < .05$) than both the unsutured, dim light, and the lid-sutured, dark reared groups. The effect of the bright light is evident in the unsutured, bright light, and lid-sutured, bright light groups (figures 6 and 7, respectively) when compared to the unsutured, dim light and the lid-sutured, dark reared groups (figures 8 and 9 respectively). There was no appatent difference in the damage to the outer segments and outer nuclear layer between the unsutured and lid-sutured rats raised in the bright light. The outer segments in both were almost completely degenerated, and only about one tenth (or one row) of the receptor nuclei remained (see figure 10), as compared with the unsutured, dim light and the lid-sutured, dark reared groups. Figure 11 shows the area where inner segments change to outer segments in an undamaged retina, in contrast to figure 10 which shows the remaining inner-most row of receptor nuclei and pigment epithelium, with evidence of degenerating membranous material between. The outer segments of the lid-sutured, dark reared group were significantly larger ($p < .05$) than those in any

Table 3. Mean thickness (μ) of retinal layers for each of the four conditions: UB, SB, UD, and SN. OS = outer segments, ONL = outer nuclear layer, INL = inner nuclear layer, IPL = inner plexiform layer, GCL = ganglion cell layer. (Note: the GCL measurement includes the optic nerve layer). The total measurement is not the sum of the other measurements because the inner segments and outer plexiform layer were not measured individually.

Retinal Layer	UB	SB	UD	SN
OS	1.2	1.2	26.6	31.6
ONL	12.2	8.6	58.2	61.4
INL	36.0	31.6	35.2	38.6
IPL	37.4	37.6	39.6	47.0
GCL	21.8	22.8	19.0	21.8
Total	109.6	102.6	196.0	224.6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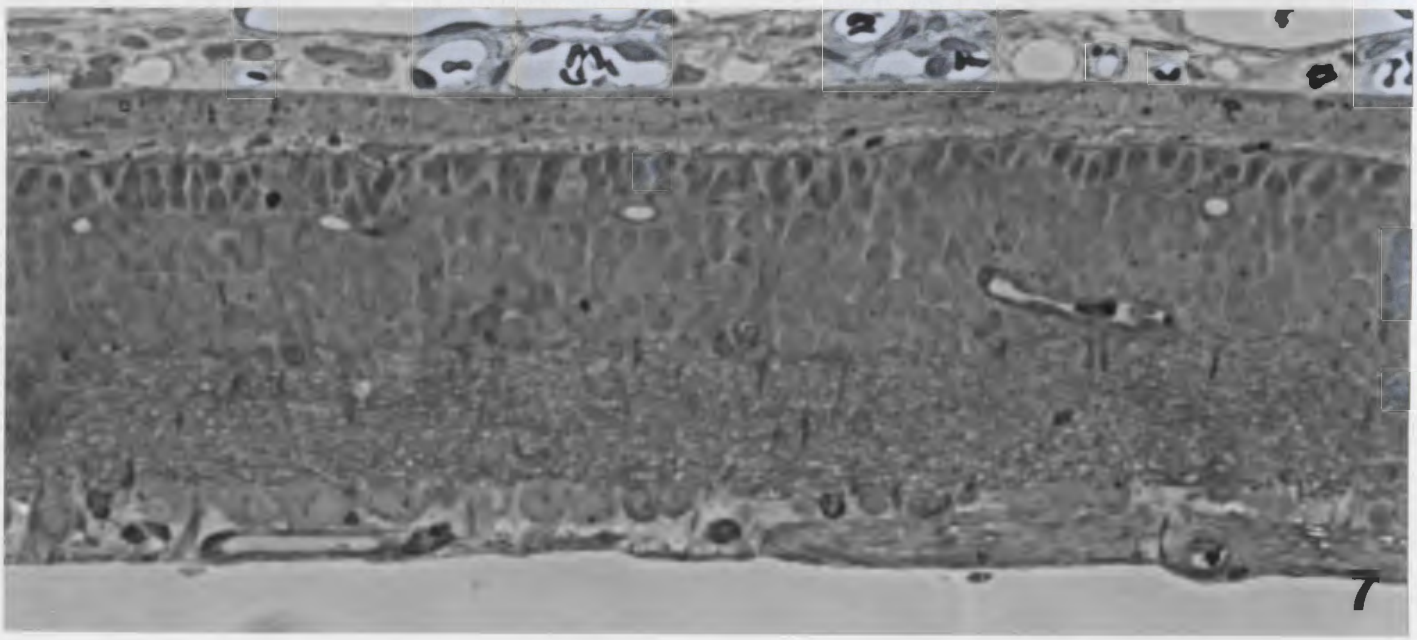
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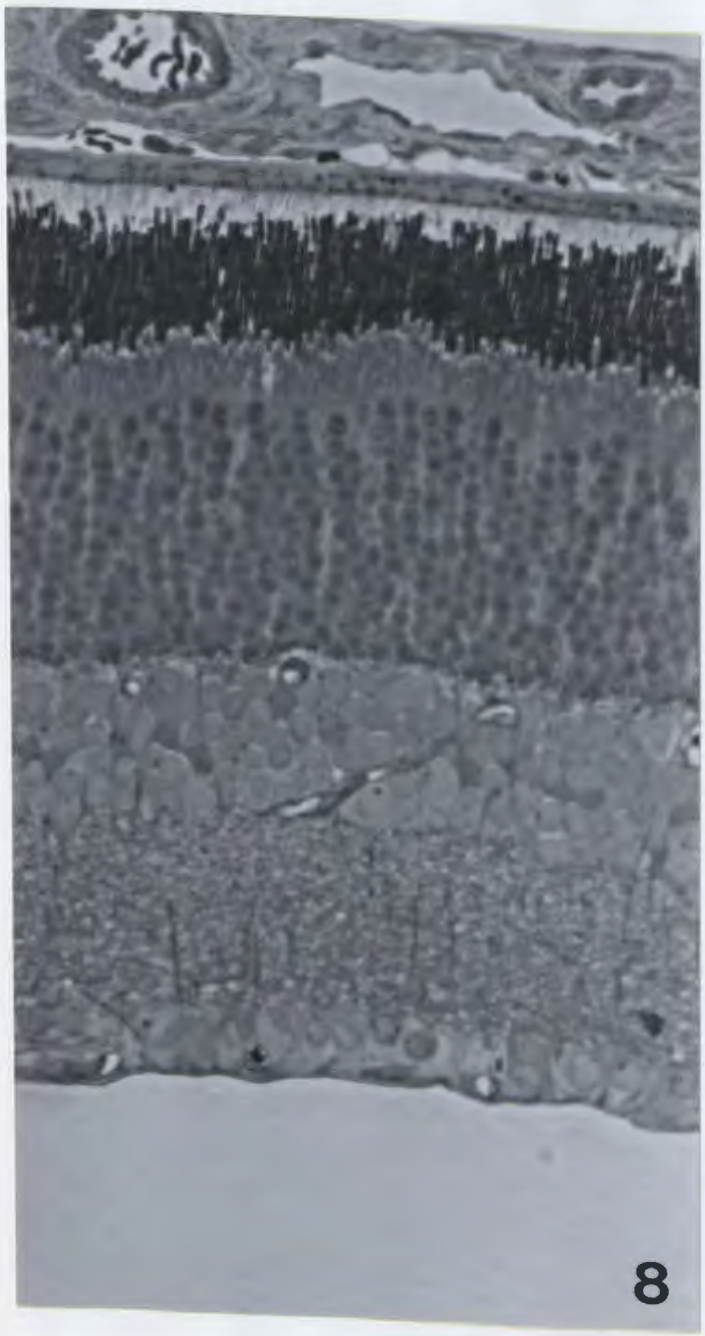
Figure 6. Light micrograph of a retina from an unsutured, bright light reared rat. Almost complete degeneration of outer segments and degeneration of about nine-tenths of the outer nuclear layer is evident by comparison to either of the undamaged retinas (figure 8 or 9). 530X

Figure 7. Light micrograph of a retina from a lid-sutured, bright light reared rat. This retina also experienced light damage, similar to that of the unsutured, bright light group (figure 6). 530X

Figure 8.. Light micrograph of a retina from an unsutured, dim light reared rat. No apparent damage to any layers of the retina was observed. 530X

Figure 9. Light micrograph of a retina from a lid-sutured, dark reared rat. The inner nuclear layer and inner plexiform layer were found to be significantly larger than those of the other conditions. 530X



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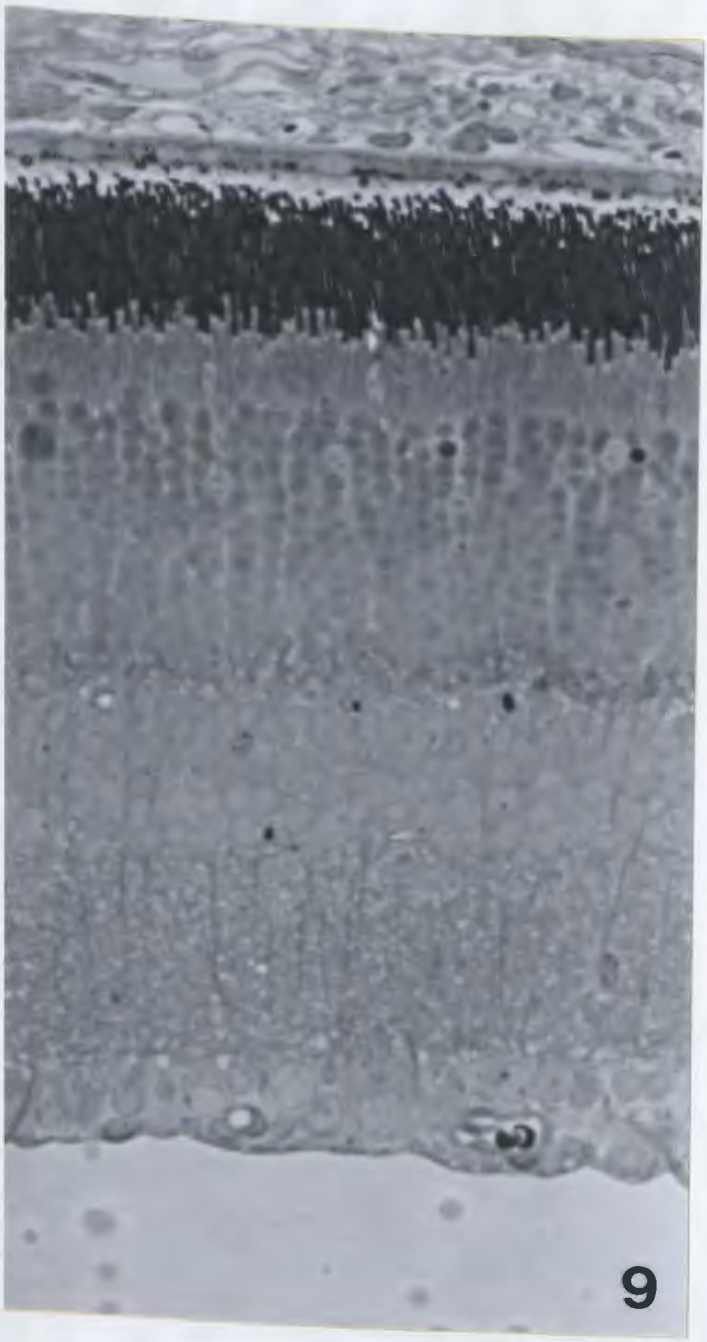
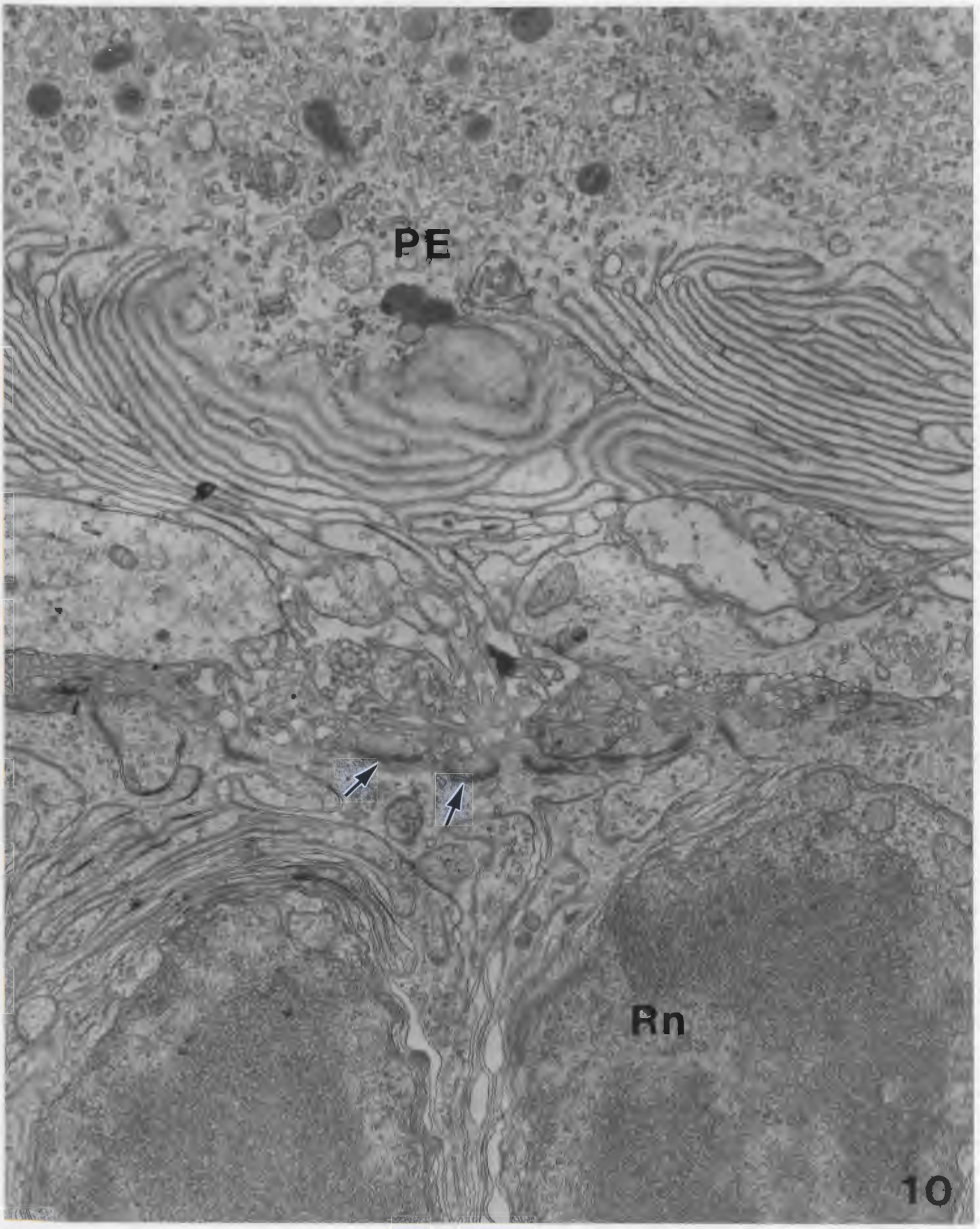


9

Figure 10. Micrograph of a light damaged retina (UB) showing the extensive receptor damage. The pigment epithelium (PE) has collapsed, and rests on the remaining degenerating tissue, previously the inner and outer segments and nine-tenths of the receptor nuclei (RN). The arrows point to the remains of the outer limiting membrane. 18960X


PE
Rn
10

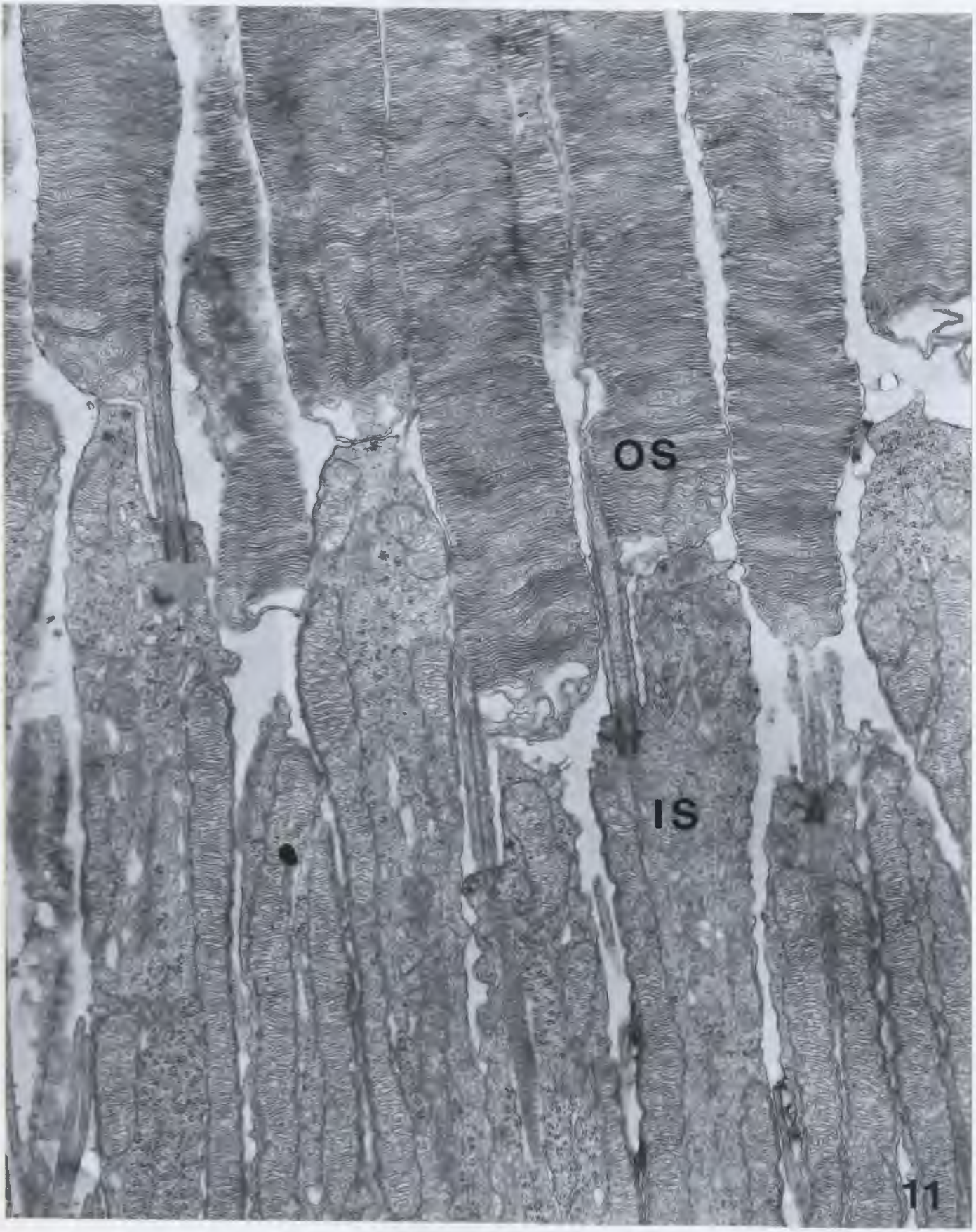

OS
IS
11

Figure 11. Micrograph of normal retina (UD) showing a portion of the intact inner segments (IS) and outer segments (OS), missing in the light damaged retinas. 19153X

other group. Measurements of the outer nuclear layer showed the unsutured, dim light, and lid-sutured, dark reared groups to be significantly larger ($p < .05$) than both the unsutured, bright light and the lid-sutured, bright light groups. The inner nuclear layer and the inner plexiform layer of the lid-sutured, dark reared group were significantly larger ($p < .05$) than those of the other groups, but there were no significant differences between the mean thicknesses of the ganglion cell layer of any of the groups.

Electron microscopy.

The two main types of synapses found in the IPL of the rat retina, the amacrine, or conventional synapse, and the bipolar, or ribbon synapse (Dubin, 1970; Sosula & Glow, 1970, 1971; Fifkova, 1972b; Leure-DuPree, 1974) were also observed in the present investigation. Amacrine synapses were typically identified by a clustering of vesicles on or adjacent to the presynaptic membrane, a membrane densification of both pre- and postsynaptic membranes, and less frequently, a post-synaptic densification of the cytoplasm and slight widening of the synaptic cleft. Examples of amacrine synapses can be seen in figures 12, 13, 14, and 15. If a cluster of vesicles was present adjacent to a membrane specialization it was judged to be a clear synapse. However, strong membrane specializations were also present with only a few vesicles; or dense clusters of vesicles were seen adjacent to membranes without strong membrane specializations. Both of these cases were also judged to be synapses. Thus, some synapses which were counted had very strong membrane specializations, but perhaps only 4-6 vesicles in the synaptic cluster, while other synapses had a minimum of membrane specialization, but had a very

Figure 12. A micrograph from a montage of the IPL of a rat retina from the unsutured, bright light group. Single arrows indicate amacrine or conventional synapses, while double arrows indicate bipolar, or ribbon synapses. Two amacrine processes (a1 and a2) are in serial configuration. 18960X

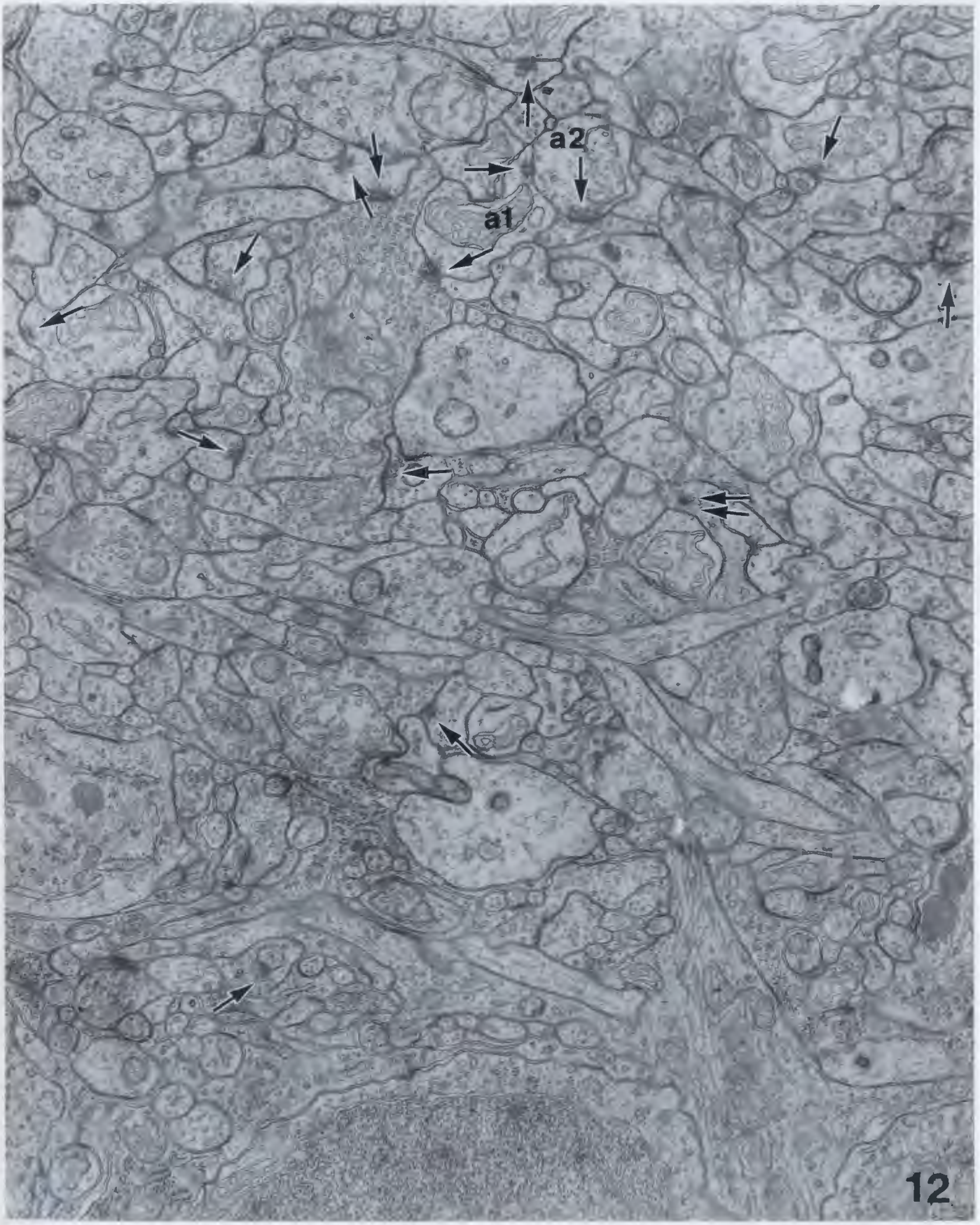
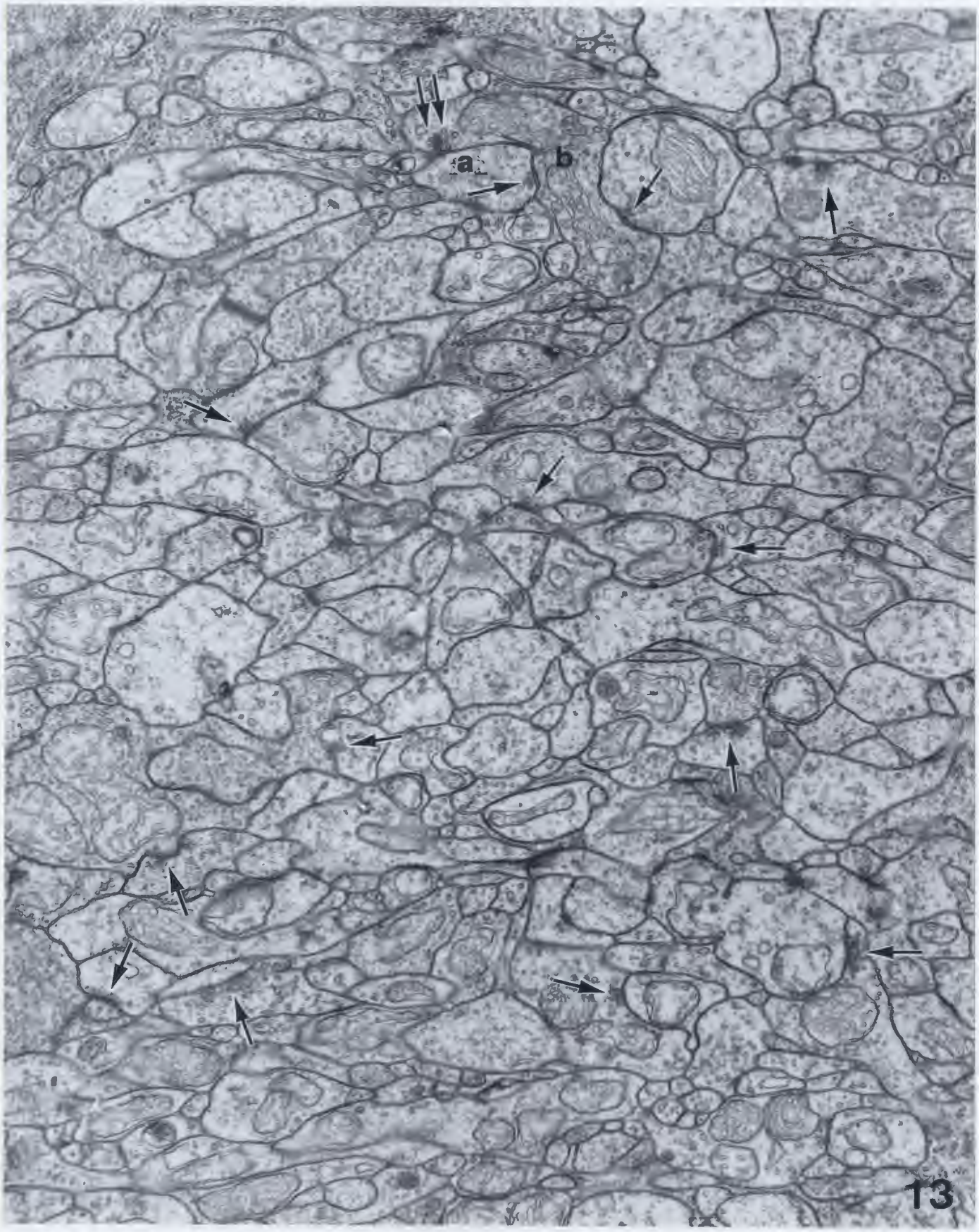

a2
a1
12

Figure 13. A micrograph from a montage of the IPL of a rat retina from the lid-sutured, bright light reared group. Single arrows indicate amacrine, or conventional synapses, while the double arrows indicate bipolar, or ribbon synapses. The amacrine process (a) forms a reciprocal synapse onto the bipolar process (b). 19196X


a
b
13

prominent cluster of synaptic vesicles. Cases in which both criteria were weak were not judged to be synapses. The identified synapses in figures 12, 13, 14, and 15 should give one a feel for the scoring.

Bipolar, or ribbon synapses were characterized by a presynaptic ribbon, surrounded by vesicles, which was usually near to and oriented toward the junction of the bipolar process and two postsynaptic processes (see figures 14 and 15). "Free-floating" ribbons were also considered as bipolar synapses, even though they were not in the usual dyad configuration (see figures 14 and 15). The free-floating ribbons create an increased bipolar incidence and account for about 25% of the bipolar total regardless of the experimental condition. Reciprocal synapses (Dowling & Boycott, 1966), which are amacrine-bipolar synapses in which the amacrine process is part of a dyad, and synapses back onto the bipolar of the dyad, were occasionally observed (see figure 13), but not counted as a separate category. Serial synapses (Dowling & Boycott, 1966; Dubin, 1970) in which an amacrine process synapses onto another amacrine process, which itself is presynaptic to some other process (as in figure 12), were counted (see Appendix D).

Synaptic incidences of all amacrine and bipolar synapses for the inner, middle, and outer layers of IPL, for each of the four rearing conditions (UB, SB, UD, and SN) are shown in table 4. The mean incidences listed in table 4 are weighted according to the area from which the incidences were calculated, and are not the average incidence of the inner, middle, and outer layers. A two-factor analysis of variance was done for each type of synapse across rearing conditions and layers of IPL (see Appendix E), and a summary is shown in table 5. For all amacrine incidences except for the amacrine-bipolar synapses,

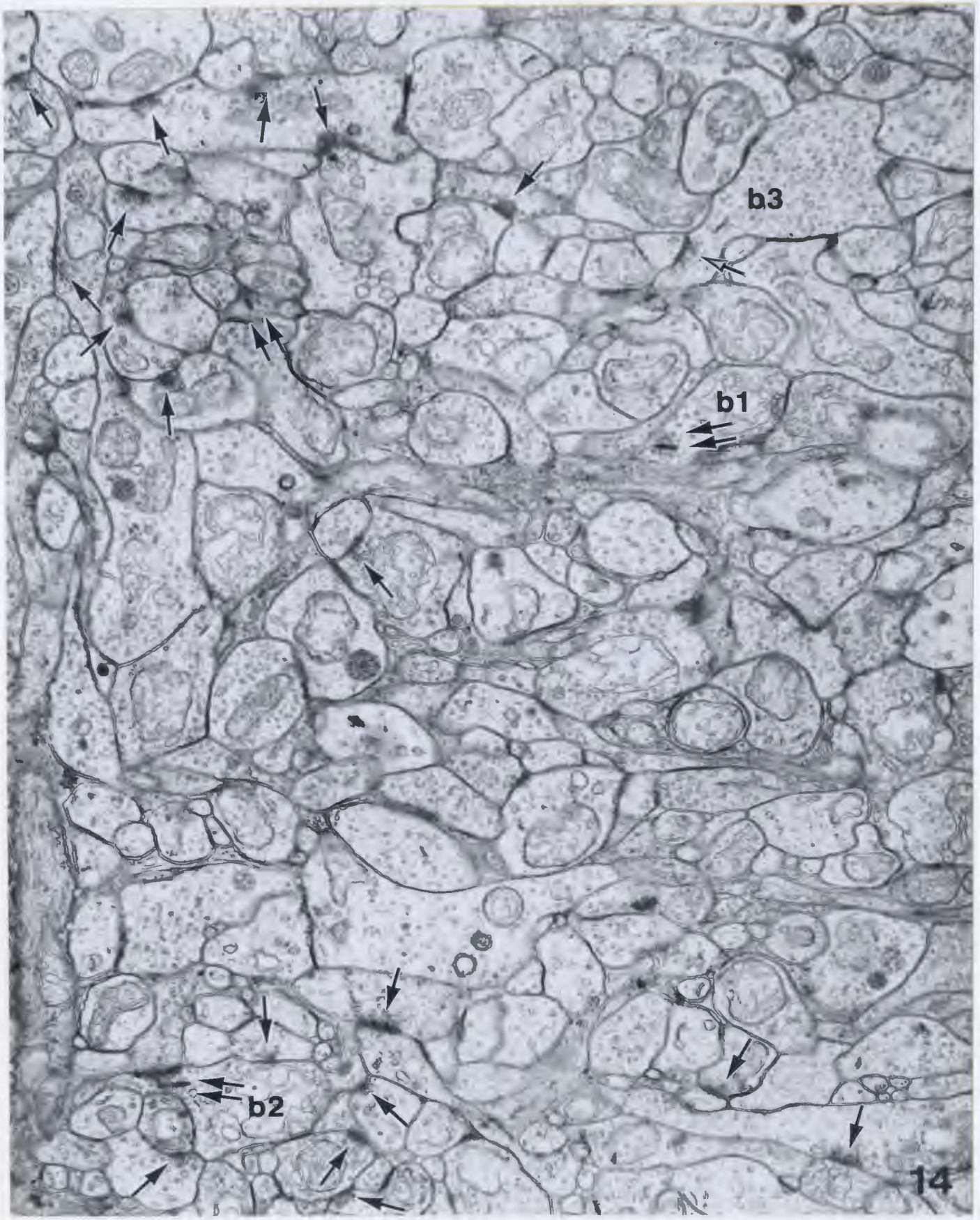

b3
b1
b2
14

Figure 14. A micrograph from a montage of the IPL of a rat retina from the unsutured, dim light group. Single arrows indicate amacrine synapses, while double arrows indicate bipolar, or ribbon synapses. The ribbons in two of the bipolar processes (b1 and b2) are free-floating; the remaining ribbon is in a dyad configuration. Conventional-looking synapses (single white arrow) in processes with bipolar-like matrices (b3) were not counted. 19196X

there was a significant difference ($p < .05$) between the inner, middle, and outer layers of the IPL. This is not unusual and in fact should be expected, as the types of synapses are not uniform throughout the layers of the IPL. No significant differences in the incidences of any bipolar synapses were observed between inner, middle, and outer layers of the IPL. With the exception of amacrine-ganglion synapses, there were no significant differences found between UB, SB, UD, and SN groups for any type of synapse in the IPL. The incidences of the amacrine-ganglion synapses were found to be significantly greater ($p < .05$) in the UB and SN groups, only in the outer third of the IPL (see table 4).

The incidence of total amacrine and total bipolar synapses for the entire IPL was also not significantly different between conditions. These data, along with the A:B ratio for each of the four rearing conditions are shown in table 6. The same incidence data is shown in figure 16 to better visualize the lack of differences between amacrine and bipolar incidences between conditions.

Table 5. Summary of the analysis of variance of the IPL. C = condition, L = layer of IPL, L3 = outer third of IPL.

		df	F	p
A/(Total)	C	3	0.3686	ns
	L	2	51.1935	.05
	CxL	6	0.3657	ns
A/A	C	3	0.0772	ns
	L	2	19.4093	.05
	CxL	6	1.0020	ns
A/B	C	3	1.5692	ns
	L	2	2.7756	ns
	CxL	6	1.0885	ns
A/G	C	3	0.4736	ns
	L	2	10.6340	.05
	CxL	6	2.6907	.05
	L1	3	0.2212	ns
	L2	3	0.7411	ns
	L3	3	5.8751	.05
A/U	C	3	1.8504	ns
	L	2	41.9443	.05
	CxL	6	0.6461	ns
Serial Amacrine	C	3	0.9483	ns
	L	2	11.4869	.05
	CxL	6	0.6258	ns
B/(Total)	C	3	1.6616	ns
	L	2	0.4149	ns
	CxL	6	0.4852	ns
B/A	C	3	1.1423	ns
	L	2	2.7978	ns
	CxL	6	1.1995	ns
B/D	C	3	0.2045	ns
	L	2	1.1245	ns
	CxL	6	1.1995	ns
B/U	C	3	1.1084	ns
	L	2	1.1814	ns
	CxL	6	0.4160	ns

Synapse	Layer of IPL	UB	SB	UD	SN
A/(Total)	INNER	.1045	.1113	.1048	.0992
	MIDDLE	.1745	.1984	.1658	.1821
	OUTER	.1635	.1674	.1514	.1585
	WEIGHTED $\bar{X}$	.1470	.1593	.1386	.1474
A/A	INNER	.0178	.0185	.0193	.0170
	MIDDLE	.0377	.0318	.0281	.0357
	OUTER	.0236	.0255	.0283	.0306
	WEIGHTED $\bar{X}$	.0267	.0252	.0246	.0281
A/B	INNER	.0257	.0246	.0268	.0230
	MIDDLE	.0238	.0159	.0227	.0278
	OUTER	.0255	.0124	.0182	.0197
	WEIGHTED $\bar{X}$	.0245	.0179	.0215	.0237
A/G	INNER	.0172	.0174	.0168	.0135
	MIDDLE	.0270	.0254	.0330	.0294
	OUTER	.0308	.0165	.0151	.0330
	WEIGHTED $\bar{X}$	.0248	.0199	.0224	.0259
A/U	INNER	.0441	.0595	.0412	.0457
	MIDDLE	.0871	.1166	.0819	.0892
	OUTER	.0793	.1085	.0894	.0752
	WEIGHTED $\bar{X}$	.0697	.0950	.0698	.0700
B/(Total)	INNER	.0264	.0253	.0246	.0256
	MIDDLE	.0258	.0265	.0304	.0226
	OUTER	.0261	.0219	.0287	.0198
	WEIGHTED $\bar{X}$	.0261	.0245	.0267	.0228
B/A	INNER	.0035	.0019	.0036	.0043
	MIDDLE	.0044	.0017	.0035	.0026
	OUTER	.0012	.0021	.0031	.0015
	WEIGHTED $\bar{X}$	.0031	.0024	.0035	.0028
B/D	INNER	.0053	.0039	.0025	.0039
	MIDDLE	.0008	.0030	.0042	.0025
	OUTER	.0020	.0017	.0035	.0050
	WEIGHTED $\bar{X}$	.0026	.0029	.0035	.0040
B/U	INNER	.0175	.0185	.0185	.0175
	MIDDLE	.0206	.0221	.0227	.0175
	OUTER	.0230	.0182	.0221	.0137
	WEIGHTED $\bar{X}$	.0202	.0197	.0210	.0161

Table 4. Comparison of the synaptic incidences in the IPLs of unsutured, bright light reared (UB), lid-sutured, bright light reared (SB), unsutured, dim light reared (UD), and lid-sutured, dark reared (SN) rats. A/(total) = total amacrine synapses, A/A = amacrine-amacrine synapses, A/B = amacrine-bipolar, A/G = amacrine-ganglion, A/U = amacrine-undefined, B/A = bipolar-2 amacrines, B/G = bipolar-ganglion, amacrine, B/U = bipolar-2 undefined (free-floating ribbons included in this category). The means shown here are weighted according to the area from which individual incidences were calculated.

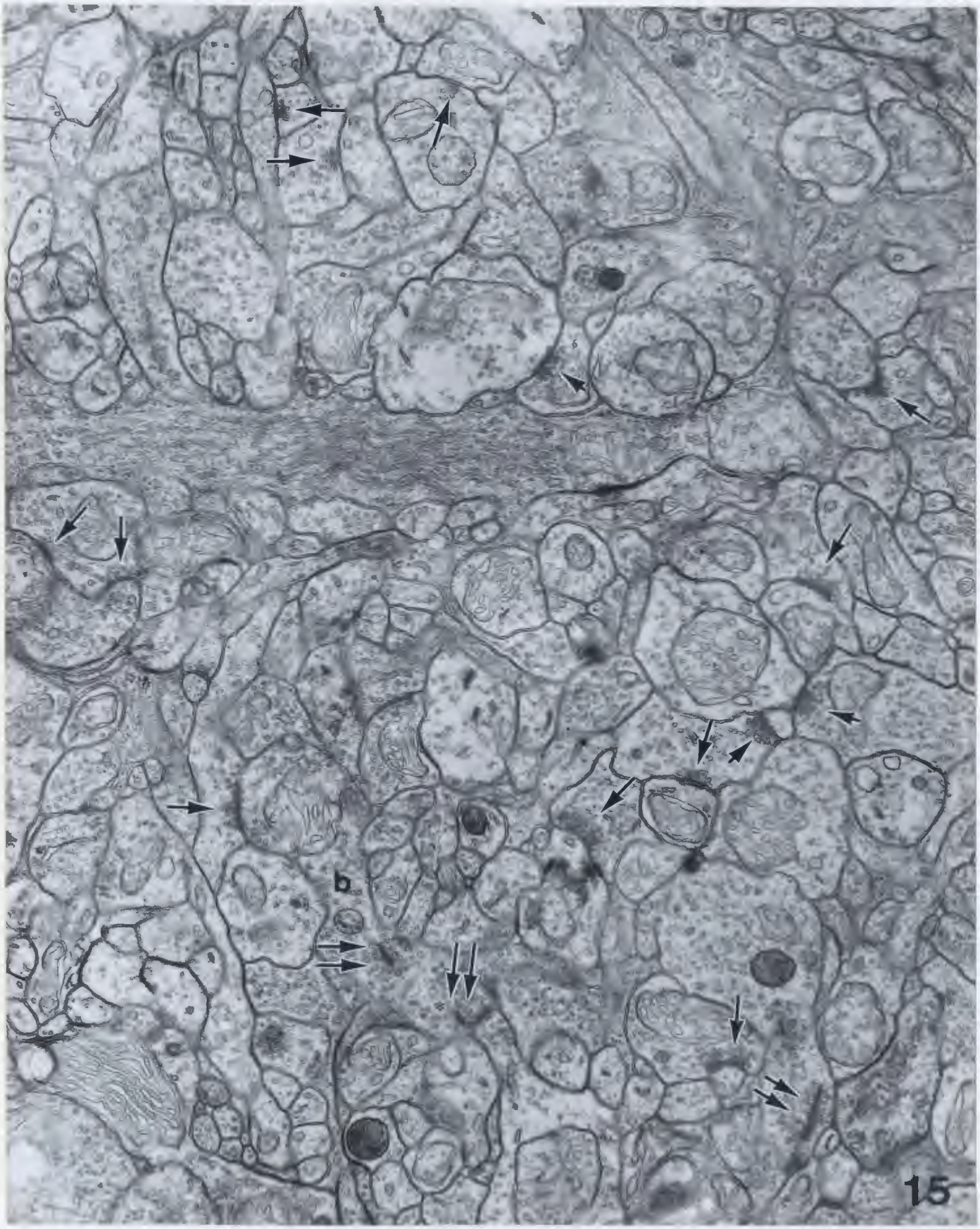
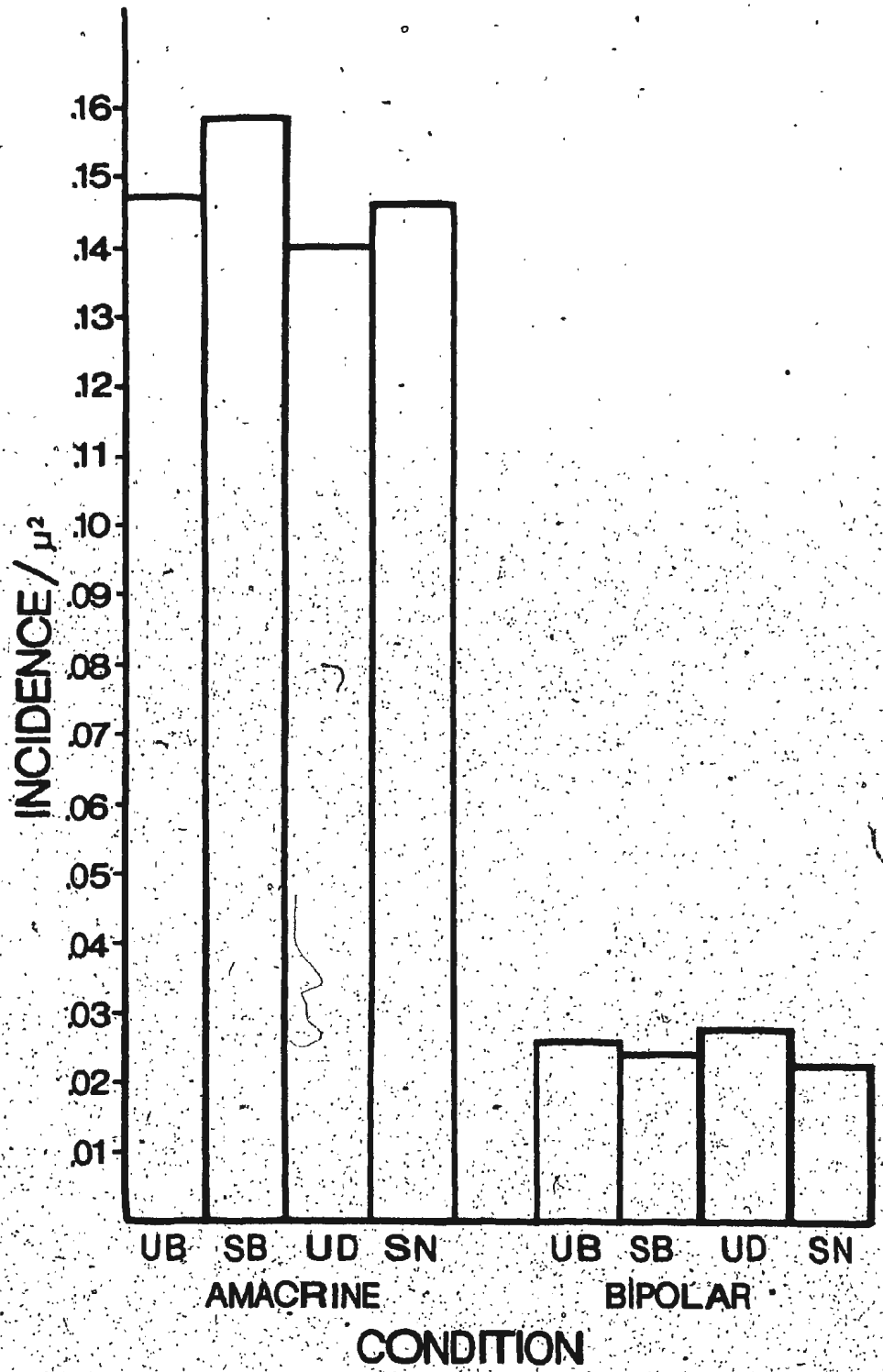

b
15

Figure 15. A micrograph from a montage of the IPL of a rat retina from the lid-sutured, dark reared group. Single arrows indicate amacrine synapses, while double arrows indicate bipolar synapses. The bipolar process (b) contains a free-floating ribbon and a ribbon in dyad configuration (*). 18895X

Table 6. Incidences of total amacrine and bipolar synapses for the entire IPL, and the A:B ratios, for the four conditions: unsutured, bright light reared (UB), lid-sutured, bright light reared (SB), unsutured, dim light reared (UD), lid-sutured, dark reared (SN).

	Amacrine Incidence/μ^2	Bipolar Incidence/μ^2	A:B
UB	.1475	.0261	5.7
SB	.1590	.0244	6.5
UD	.1407	.0279	5.0
SN	.1466	.0227	6.5

Figure 16. Graph of the incidence of total amacrine and bipolar synapses in the IPL. Note the lack of differences across conditions.


.16
.15
.14
.13
.12
.11
.10
.09
.08
.07
.06
.05
.04
.03
.02
.01
INCIDENCE / μ²
UB SB UD SN
UB SB UD SN
AMACRINE
BIPOLAR
CONDITION

Discussion

Light microscopy.

Continuous bright light (1960 lux for 8 weeks) caused extensive damage to the outer retinal layers of both unsutured and lid-sutured rats, as seen in figures 6 and 7, and in the measurements of OS and ONL (table 3). The degeneration observed in the present study is in agreement with the earlier studies on the deleterious effects of continuous light even at normal and sub-normal intensities (Bennett, et al., 1973a, 1973b; Gorn & Kuwabara, 1967; Anderson & O'Steen, 1972; O'Steen & Anderson, 1971, 1972), and alternating periods of bright light and darkness (Shear, et al., 1973; Fifkova, 1973). The damage to the OS and ONL of the UB group is understandable, but the similarly extensive damage to the SB group is confusing. The intensity of light for the SB group was equal to that of the UD group (9.8 lux), and the retinas of the UD group were in all respects normal. Both groups were kept in continuous light, but those in the UD group could still close their eyes, and in effect could cycle the light, whereas those in the SB group could not cycle the light. It must be assumed that even very low intensity light (9.8 lux) will cause severe retinal damage if continuous. The extent of damage produced by 9.8 lux is the same as that produced by 1960 lux. A comparison of figures 6 and 7 with figures 8 and 9 shows that the only thing that remains of the normally thick receptors is one scanty row of receptor nuclei (see figure 10). The damaged retinas have collapsed, and degenerating membranes and phagocytic tissue separate the pigment epithelium from the remaining receptor nuclei of the ONL.

The greater length of outer segments found in the SN group is probably explained by a reduced phagocytosis of the receptor disks in the dark. The ONL of both the UD and SN groups were similar, indicating that the ONL of the SN group was normal. The finding of lafger INL and IPL in the SN retinas is supported by Cragg (1969), but is opposed to Weiskrantz (1958) and Rasch et al. (1961), who have observed a decrease in thickness of IPL after light deprivation, and contrary to Sosula & Glow (1971) and Fifkova (1972b) who find no change in thickness of IPL due to light deprivation.

Electron microscopy.

The state of the literature on the IPL of rat retina is very inconsistent. Investigators cannot agree on the incidences of amacrine synapses and bipolar synapses in the IPLs even of normal or control animals (see table 1). As suggested earlier, methodological and criterion differences may make a significant contribution to the discrepancies. Dubin (1970) fixed by immersion in osmium as did Sosula & Glow (1970, 1971), yet the two studies had quite different incidences of bipolar synapses. Fifkova (1972b) perfused with glutaraldehyde, and post-fixed in osmium. Her results are very different from both Dubin (1970) and Sosula & Glow (1970, 1971). Sosula & Glow (1970, 1971) used a double staining method (uranyl acetate followed by lead citrate), while Fifkova (1972b) used lead citrate alone. Dubin (1970) did not specify whether lead citrate was used alone, or uranyl acetate used prior to it on the rat retinas he examined. In Dubin's (1970) study, micrographs were taken at 4,000-6,000 X and finally printed at 15,000-20,000 X, and in Fifkova's (1972b) study, micrographs were taken at 3,800 X and printed at a final magnification of 26,000 X,

while Sosula & Glow (1970, 1971) photographed the IPL at 10,000 X and printed at a final magnification of 15,000 X. It is a strong possibility, in the cases of Dubin (1970) and Fifkova (1972b), that much of the detail needed to identify some synapses is lost during the enlargement process. It must also be remembered that Sosula & Glow (1970, 1971) used adult hooded rats, and Fifkova (1972b) used young (10 weeks old at time of comparison) albino rat pups. Dubin (1970) did not specify the age of the albino rats he used.

A quite recent study (not mentioned in table 1) by Leure-DuPree (1974) used adult albino rats. The retinas were fixed by perfusion with glutaraldehyde, then post-fixed in osmium, and double stained with uranyl acetate followed by lead hydroxide. The print magnification was given, but not the photographic magnification. The criterion for synaptic identification was similar to that of Dowling & Boycott (1966). He obtained an A:B ratio of 3.0--close to that found by Dubin (1970)--but there is a big difference in methodology between Leure-DuPree (1974) and the other studies.

The large discrepancies between the studies are difficult (almost impossible) to attribute to any one of the methodological or criterion differences, and are almost certainly due to a combination of all the factors for any one study.

With such large discrepancies (especially those between Sosula & Glow (1970, 1971) and Fifkova (1972b) of amacrine and bipolar incidences found in normal rats, what is to be expected when electron microscopy is used to investigate the effects of light deprivation or light damage on the IPL?

As seen from table 2, Sosula & Glow (1971) report very large

The inconsistency of the previous investigations of IPL of normal (and of light damaged and light deprived) rat retina suggests the need for one study to investigate the effects of light damage and light deprivation. A study in which all retinas are fixed with the same procedure, the same fixative, at the same time, where the microtomy, microscopy, and scoring are done blind, where montages are used with the magnification determined separately for each, and where one criterion is used for all experimental conditions. This study did just that.

The significant difference found between inner, middle, and outer layers of IPL for all but amacrine-bipolar synapses is not a surprising, or even unusual result, because frequencies of the different types of synapses are known to vary in the different layers of the IPL (Sosula & Glow, 1971).

Contrary to the findings of Sosula & Glow (1971) and Fifkova (1972b, 1973), the results of the present investigation indicate that (with the exception of amacrine-ganglion synapses) there are no significant differences in the incidences of any type of synapse in the IPL due to light deprivation or light damage. The incidences in table 1, shown graphically in figure 16, are very consistent between groups. Table 7 shows the changes in total amacrine and total bipolar incidences expressed as per cent of the control retinas (the UD group in the present study). Sosula & Glow (1971) did not study light damaged retinas, while Fifkova (1973) found that the total amacrine incidence increased by 25.4% with light damage, but did not report any significance tests on it. A non-significant 6.1% increase was observed in the present study. The total bipolar incidence decreased

increases in incidences of amacrine synapses in light deprived rats, whereas those found by Fifkova (1972b), although significant in some cases, are much smaller. The most inconsistent findings are the changes in amacrine-ganglion synapses. Sosula & Glow (1971) find a 3.5 fold increase, whereas Fifkova (1972b) finds a 0.9 fold decrease. Such a strong increase as that found by Sosula & Glow (1971) is debatable, and very much in contrast to that found by Fifkova (1972b). Not only did Sosula & Glow (1971) have a small sample size for comparison, but also used the type of fixation which would make differentiation of ganglion cell dendrites from amacrine processes less clear. Most certainly, Fifkova (1972b) would be better able to identify the ganglion cell dendrites by their matrices, and thus have a more accurate count. Her results are opposite to those of Sosula & Glow (1971). I suspect that the large results obtained by Sosula & Glow (1971) are probably due to artifact (small sample size, perhaps different areas, or even criterion differences).

Some of the problems of comparisons using electron microscopy have been mentioned in the Introduction, but the main point is that consistency of methodology should be sought by the investigator. Consistent results are very difficult to obtain in electron microscopy, and thus significant differences may be due to artifact rather than true differences. Sosula & Glow (1971) compared only one montage of normal IPL with one montage of light deprived IPL (also sets of 5 micrographs biased for bipolars), while Fifkova (1972b) compared "random" micrographs throughout the IPL of 7 deprived retinas with 3 controls. These types of comparisons are not as sound as using many large montages covering the entire depth of IPL.

Table 7. Comparisons of the changes in synaptic incidences (expressed as % of controls) of total amacrine and total bipolar synapses due to light damage and light deprivation. Fifkova's light damaged data is from Fifkova (1973), her light deprived data is from Fifkova (1972b). * = no statistics reported.

		Light Damaged		Light Deprived	
Amacrine	Sosula & Glow	-		+137.0	.001
	Fifkova	+25.4	*	+33.5	*
	Chernenko	+6.1	ns	+6.3	ns
Bipolar	Sosula & Glow	-		+49.2	ns
	Fifkova	-11.4	*	-18.7	.02
	Chernenko	-2.2	ns	-14.6	ns

by 11.4% (again no statistics reported) in Fifkova's (1973) study, while a non-significant 2.2% decrease was observed in the present study. The direction of change due to light damage in the present study was similar to that of Fifkova's (1973), but not nearly as strong.

Sosula & Glow (1971) observed a 137.0% increase ($p < .001$) in total amacrine incidence after light deprivation, Fifkova (1972b) observed a 33.5% increase (no statistics were reported, but she is in agreement with Sosula & Glow (1971) "of a significant increase in amacrine contacts in the IPL of deprived rat's eyes." (Fifkova, 1972b)). A non-significant 6.3% increase was observed in the present investigation. Sosula & Glow (1971) found a non-significant 49.2% increase in total bipolar incidence, Fifkova (1972b) found an 18.7% decrease ($p < .02$), while the present study found a non-significant 14.6% decrease. The present study agrees with the other two studies as to the direction of change of total amacrine incidence, but not to the magnitude and certainly not to the significance. Findings on the total bipolar incidence are in agreement with Fifkova (1972b) on the direction of change, but not on significance, and are not in agreement with direction or significance with Sosula & Glow (1971).

The only significant finding in the present investigation was an interaction effect for amacrine-ganglion synapses due to both light deprivation and light damage (see table 5 and Appendix F). Amacrine-ganglion synapses were significantly greater ($p < .05$) in the UD and SN conditions in <u>only</u> the outer third of the IPL, as seen from table 4. It was in this category of amacrine-ganglion synapses in which Sosula & Glow (1971) found a 245.7% increase ($p < .001$), Fifkova

(1972b) found a non-significant 1.5% decrease, while in the present study a 15.6% increase ($p < .05$) was found after light deprivation. The size of the increase found by Sosula & Glow (1971) is questioned, even though the present findings are in a similar direction, and significant. The type of fixation used by Fifkova (1972b) was probably better suited for identifying ganglion processes than the present study, and certainly better than that of Sosula & Glow (1971). Fifkova (1973) found a significant (no statistics reported) 154.5% increase in amacrine-ganglion synapses after light damage, while the present study found a significant 10.7% increase ($p < .05$) in the outer third of the IPL only.

Previous investigators (Sosula & Glow, 1971; Fifkova, 1972b, 1973) have suggested that the retina possesses an amazing plasticity which appears as a dramatic change, mainly in the amacrine synapses in the IPL after light deprivation or light damage. Results from the present investigation have shown that retinal plasticity, as a consequence of light deprivation or light damage is certainly not as dramatic as was initially thought, and, if it exists, the amacrine-ganglion synapses are probably the only synapses to reflect this plasticity.

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APPENDIX A

SOLUTIONS FOR FIXATION AND PROCEDURAL SCHEDULE

APPENDIX A - TABLE 1

Solutions for Fixation

2.5% glutaraldhyde (store in cold < 3 weeks)

42.5 ml water
5.0 ml 25% glutaraldhyde
0.7435 g Na cadodylate
2.25 g sucrose

Wash (store in cold < 3 weeks)

50 ml water
0.716 g Na cacodylate
2.25 g sucrose

Veronal acetate buffer (50 ml) (store in cold < 4 weeks)

36 ml water
0.578 g Na Veronal
0.382 g Na acetate ($3.H_2O$)
12 ml 0.1 N HCl
2.25 g sucrose
2.0 ml 1% $CaCl_2$ (anhydrose)

4% OsO_4 (store in cold until changes color-darker brown)

25 ml water
1 g OsO_4

Luft Hard Epon (40:60) (for 20 ml)

Epon	9.41 ml
DDSA	4.94 ml
NMA	5.65 ml
DMP-30	0.3 ml

(from Polysciences)

APPENDIX A - TABLE 2

Fixation Procedure

1. Drop fresh tissue into cold 2% Veronal acetate buffered OsO_4. Leave for 90 minutes.
2. Wash for 5 minutes on ice.
3. Cold 2.5% glutaraldhyde for 60 minutes.
4. Rinse in distilled water for 1 minute.
5. Dehydrate in ETOH

   50%-5 minutes

   70%-10 minutes

   85%-10 minutes

   95%-10 minutes

   100%-3 changes over 20-60 minutes with last change to Absolute.
6. Propylene oxide - 3 changes over 20 minutes.
7. 50:50 propylene oxide-Epon for 60 minutes.
8. 20:80 propylene oxide-Epon overnight.
9. 100% Epon in rubber mould for 2 days in 50°C. oven.

APPENDIX B

THICKNESS (μ) OF RETINAL LAYERS TAKEN FROM LIGHT MICROGRAPHS

APPENDIX B - TABLE I

Thickness (μ) of light micrographs of retinal layers

Retinal Layer	UB	SB	UD	SN
	3	0	27	32
	1	2	28	32
OS	0	2	25	35
	0	0	25	30
	2	2	28	29
	20	5	58	58
	8	10	58	60
ONL	8	10	60	60
	10	6	55	65
	15	12	60	64
	40	30	38	38
	33	34	35	35
INL	33	32	34	40
	33	30	34	40
	36	32	35	40
	42	34	41	45
	33	40	39	47
IPL	39	32	37	48
	30	35	42	50
	43	47	39	45
	20	16	18	22
	19	18	16	23
GCL	25	18	16	19
	18	22	25	25
	27	40	20	20
	135	87	200	215
	97	105	193	225
Total	103	97	192	228
	93	94	195	235
	120	130	200	220

APPENDIX C

ANOVA AND NEWMAN-KEULS

ON RETINAL LAYERS FROM LIGHT MICROGRAPHS

APPENDIX C - TABLE 1

Outer Segments (OS)

Source	df	SS	MS	F	p
Btwn	3	3954.55	1318.1833	502.17	<.01
Within	16	42.00	2.625		
Total	19	3996.55			

Newman-Keuls: df=16 Sw^2=2.625 $S\bar{x}=(Sw^2/n)^{\frac{1}{2}}$

	SB	UB	UD	SN
SB	0	0	35.055	41.956
UB			35.055	41.956
UD				6.901
SN				

Comparisons:

SN-SB	<.05
SN-UB	<.05
SN-UD	<.05
UD-SB	<.05
UD-UB	<.05

APPENDIX C – TABLE 2

Outer Nuclear Layer (ONL)

Source	df	SS	MS	F	p
Btwn	3	12259.8	4086.6	333.6	<.01
Within	16	196.0	12.25		
Total	19	12455.8			

Newman-Keuls: df=16 Sw^2=12.25 $\bar{Sx}=(Sw^2/n)^{\frac{1}{2}}$

	SB	UB	UD	SN
SB		2.300	31.688	33.733
UB			29.388	31.433
UD				2.044
SN				

Comparisons:

SN-SB	<.05
SN-UB	<.05
UD-SB	<.05
UD-UB	<.05

APPENDIX C - TABLE 3

Inner Nuclear Layer (INL)

Source	df	SS	MS	F	P
Btwn	3	122.60	40.8667	8.2559	<.01
Within	16	79.20	4.95		
Total	19	201.80			

Newman-Keuls: df=16 S_w^2=4.95 $S_{\bar{x}}=(S_w^2/n)^{\frac{1}{2}}$

	SB	UB	UD	SN
SB		3.417	3.618	7.035
UB			0.201	3.618
UD				3.417
SN				

Comparisons:

SN-SB	<.05
SN-UD	<.05
SN-UB	<.05

APPENDIX C - TABLE 4

Inner Plexiform Layer (IPL)

Source	df	SS	MS	F	p
Btwn	3	305.2	101.73	5.2915	<.01
Within	16	307.6	19.225		
Total	19	612.8			

Newman-Keuls: df=16 Sw^2=19.225 $S\bar{x}=(Sw^2)^{\frac{1}{2}}$

	UB	SB	UD	SN
UB		.102	1.122	4.896
SB			1.020	4.794
UD				3.774
SN				

Comparisons:

SN-UD	<.05
SN-UB	<.05
SN-SB	<.05

APPENDIX C – TABLE 5

Ganglion Cell Layer (GCL)

Source	df	SS	MS	F	p
Btwn	3	40.15	13.3833	.4037	ns
Within	16	530.40	33.1500		
Total	19	570.55			

APPENDIX D

THE TABULATED SYNAPTIC COUNTS

FOR EACH MONTAGE OF IPL

APPENDIX D - TABLE 1

Tabulated Synaptic Count for Montage UB3R (C3)

Number of Synapses

Synapse	Inner 1/3	Middle 1/3	Outer 1/3	IPL
Amacrine				
Total	55	86	68	209
A	12	19	12	43
B	12	22	12	46
C	7	11	10	28
U	24	34	34	92
Bipolar				
Total	25	11	11	47
A	2	2	0	4
D	7	0	0	7
U	16	9	11	36
Serial Amacrine	2	6	2	10
Area (μ^2)	574.6	574.6	574.6	1723.8

APPENDIX D – TABLE 2

Tabulated Synaptic Count for Montage UB5R (B1)

Number of Synapses

Synapse	Inner 1/3	Middle 1/3	Outer 1/3	IPL
Amacrine				
Total	66	100	91	257
A	15	22	14	51
B	7	4	16	27
G	19	17	20	56
U	25	57	41	123
Bipolar				
Total	14	10	16	40
A	4	4	2	10
D	4	1	4	9
U	6	5	10	21
Serial Amacrine	1	4	3	8
Area (μ^2)	515.5	515.5	515.5	1546.5

APPENDIX D - TABLE 3

Tabulated Synaptic Count for Montage UB3R (A3)

Number of Synapses

Synapse	Inner 1/3	Middle 1/3	Outer 1/3	LPL
Amacrine				
Total	50	90	78	218
A	3	15	7	25
B	22	12	12	46
G	6	16	19	41
U	19	47	30	96
Bipolar				
Total	5	11	10	26
A	2	1	0	3
D	0	0	1	1
U	3	10	9	22
Serial Amacrine	2	2	0	4
Area (μ^2)	466.9	466.9	466.9	1400.7

APPENDIX D - TABLE 4

Tabulated Synaptic Count for MontageUB1L (E3)

Number of Synapses

Synapse	Inner 1/3	Middle 1/3	Outer 1/3	IPL
Amacrine				
Total	48	82	107	237
A	8	29	21	58
B	12	11	14	37
G	6	8	11	25
U	22	34	61	117
Bipolar				
Total	11	19	13	43
A	1	3	1	5
D	1	0	0	1
U	9	16	12	37
Serial Amacrine	0	6	5	11
Area (μ^2)	482.3	482.3	482.3	1446.9

APPENDIX D - TABLE 5

Tabulated Synaptic Count for Montage UB2L (D2)

Number of Synapses

Synapse	Inner 1/3	Middle 1/3	Outer 1/3	IPL
Amacrine				
Total	40	72	58	170
A	7	9	5	21
B	9	8	9	26
G	5	14	15	34
U	19	41	29	89
Bipolar				
Total	12	12	14	38
A	0	1	0	1
D	2	1	0	3
U	10	10	14	34
Serial Amacrine	0	1	0	1
Area (μ^2)	434.2	434.2	434.2	1302.6

APPENDIX D - TABLE 6

Tabulated Synaptic Count for Montage SB1R (E1)

Number of Synapses

Synapse	Inner 1/3	Middle 1/3	Outer 1/3	IPL
Amacrine				
Total	62	76	53	191
A	9	11	9	29
B	16	13	5	34
G	8	12	3	23
U	29	40	36	105
Bipolar				
Total	16	10	14	40
A	1	0	2	3
D	3	1	0	4
U	12	9	12	33
Serial Amacrine	1	2	0	3
Area (μ^2)	540.6	540.6	540.6	1621.8

APPENDIX D - TABLE 7

Tabulated Synaptic Count for Montage SB2L (D3)

Synapse	Number of Synapses			
	Inner 1/3	Middle 1/3	Outer 1/3	IPL
Amacrine				
Total	39	59	78	176
A	10	8	12	30
B	6	8	4	18
C	3	8	9	20
U	20	35	43	98
Bipolar				
Total	13	13	7	33
A	1	3	4	4
D	1	0	1	2
U	11	10	6	5
Serial Amacrine	3	0	2	5
Area (μ^2)	454.4	454.4	454.4	1363.2

APPENDIX D - TABLE 8

Tabulated Synaptic Count for Montage SB2R (01)

Number of Synapses

Synapse	Inner 1/3	Middle 1/3	Outer 1/3	IPL
Amacrine				
Total	79	138	94	311
A	9	17	8	34
B	9	15	8	32
G	14	17	15	46
U	47	89	63	199
Bipolar				
Total	16	14	13	43
A	2	0	0	2
D	1	3	1	5
U	13	11	12	36
Serial Amacrine	3	8	4	15
Area (μ^2)	522.1	522.1	522.1	1566.3

APPENDIX D - TABLE 9

Tabulated Synaptic Count for Montage SB5L (B3)

Synapse	Number of Synapses			
	Inner 1/3	Middle 1/3	Outer 1/3	IPL
Amacrine				
Total	49	126	103	278
A	11	30	18	59
B	3	12	9	24
G	7	6	4	17
U	28	78	72	178
Bipolar				
Total	9	18	13	40
A	1	0	1	2
D	1	0	1	2
U	7	18	11	36
Serial Amacrine	3	8	6	17
Area (μ^2)	529.4	529.4	529.4	1588.2

APPENDIX D - TABLE 10

Tabulated Synaptic Count for Montage SB3R (A4)

Number of Synapses

Synapses	Inner 1/3	Middle 1/3	Outer 1/3	IPL
Amacrine				
Total	57	117	82	256
A	8	17	15	40
B	7	16	5	28
C	13	23	9	45
U	29	61	53	143
Bipolar				
Total	8	14	8	30
A	0	1	2	3
B	4	4	1	9
U	4	9	5	18
Serial Amacrine	1	5	6	12
Area (μ^2)	507.7	533.4	426.0	1457.1

APPENDIX D - TABLE 11

Tabulated Synaptic Count for Montage UD1L (E4)

Number of Synapses

Synapse	Inner 1/3	Middle 1/3	Outer 1/3	IPL
Amacrine				
Total	58	84	72	214
A	14	17	17	48
B	15	11	8	34
G	5	13	8	24
U	23	43	41	108
Bipolar				
Total	7	16	15	38
A	0	2	1	3
D	0	0	1	1
U	7	14	13	34
Serial Amacrine	2	2	1	5
Area (μ^2)	434.1	434.1	434.1	1302.3

APPENDIX D - TABLE 13

Tabulated Synaptic Count for Montage UD2R (C2)

Number of Synapses

Synapse	Inner 1/3	Middle 1/3	Outer 1/3	IPL
Amacrine				
Total	51	78	66	195
A	7	12	15	34
B	18	16	8	42
G	10	10	4	24
U	16	40	39	95
Bipolar				
Total	14	7	10	31
A	1	1	1	3
D	2	1	2	5
U	11	5	7	23
Serial Amacrine	1	5	4	10
Area (μ^2)	400.3	400.3	400.3	1200.9

APPENDIX D - TABLE 12

Tabulated Synaptic Count for Montage UD2L (D4)

Synapse	Number of Synapses			
	Inner 1/3	Middle 1/3	Outer 1/3	IPL
Amacrine				
Total	51	96	92	239
A	13	22	14	49
B	14	8	13	35
G	7	15	15	37
U	17	51	50	118
Bipolar				
Total	17	23	15	55
A	4	1	1	6
D	0	3	3	6
U	13	19	11	43
Serial Amacrine	0	3	2	5
Area (μ^2)	599.3	638.3	638.3	1875.9

APPENDIX D - TABLE 14

Tabulated Synaptic Count for Montage UD5R (B2)

Number of Synapses

Synapse	Inner 1/3	Middle 1/3	Outer 1/3	IPL
Amacrine				
Total	61	118	93	272
A	10	12	15	37
B	7	10	9	26
G	13	40	7	60
U	29	56	61	146
Bipolar				
Total	9	21	15	45
A	3	3	3	9
D	1	6	3	10
U	5	12	9	26
Serial Amacrine	2	3	3	8
Area (μ^2)	568.6	586.3	552.1	1707.0

APPENDIX D - TABLE 15

Tabulated Synaptic Count for Montage UD3R (A2)

Number of Synapses

Synapse	Inner 1/3	Middle 1/3	Outer 1/3	IPL
Amacrine				
Total	38	48	58	144
A	4	9	8	21
B	10	10	8	28
G	7	10	8	25
U	17	19	34	70
Bipolar				
Total	15	14	17	46
A	2	2	2	6
D	3	2	0	5
U	10	10	15	35
Serial Amacrine	0	1	1	2
Area (μ^2)	539.1	539.1	510.2	1588.4

APPENDIX D - TABLE 19

Tabulated Synaptic Count for Montage SN8L (C4)

Number of Synapses

Synapse	Inner 1/3	Middle 1/3	Outer 1/3	IPL
Amacrine				
Total	45	101	73	219
A	9	24	14	47
B	15	16	9	40
G	7	20	19	46
U	14	41	31	86
Bipolar				
Total	15	11	5	31
A	3	1	0	4
D	1	1	2	4
U	11	9	3	23
Serial Amacrine	0	3	4	7
Area (μ^2)	546.8	546.8	546.8	1640.4

APPENDIX D - TABLE 18

Tabulated Synaptic Count for Montage SN8R (B4)

	Number of Synapses			
Synapse	Inner 1/3	Middle 1/3	Outer 1/3	IPL
<u>Amacrine</u>				
Total	55	116	92	263
A	3	7	12	22
B	11	19	13	43
G	6	12	15	33
U	35	78	52	165
<u>Bipolar</u>				
Total	10	6	9	25
A	2	2	1	5
D	1	1	2	4
U	7	3	6	16
Serial Amacrine	1	3	3	7
Area (μ^2)	505.0	505.0	505.0	1515.0

APPENDIX D - TABLE 17

Tabulated Synaptic Count for Montage SN6R (D1)

Number of Synapses

Synapse	Inner 1/3	Middle 1/3	Outer 1/3	IPL
Amacrine				
Total	42	73	63	178
A	9	9	10	28
B	12	13	8	33
G	5	7	10	22
U	16	44	35	95
Bipolar				
Total	13	10	14	37
A	3	0	0	3
D	2	2	1	5
U	8	8	13	29
Serial Amacrine	0	3	4	7
Area (μ^2)	508.4	553.9	502.2	1564.5

APPENDIX D - TABLE 16

Tabulated Synaptic Count for Montage SN3R (E2)

Number of Synapses

Synapses	Inner 1/3	Middle 1/3	Outer 1/3	IPL
Amacrine				
Total	55	68	73	196
A	9	21	17	47
B	12	5	9	26
G	8	11	11	30
U	26	31	36	93
Bipolar				
Total	12	13	6	31
A	1	1	1	3
D	1	0	0	1
U	10	12	6	28
Serial Amacrine	2	3	5	10
Area (μ^2)	450.6	450.6	450.6	1351.8

APPENDIX D – TABLE 20

Tabulated Synaptic Count for Montage SN5L (A1)

Synapse	Number of Synapses			
	Inner 1/3	Middle 1/3	Outer 1/3	IPL
Amacrine				
Total	52	125	111	288
A	13	34	27	74
B	8	22	12	42
G	8	29	33	70
U	23	40	39	102
Bipolar				
Total	15	20	18	53
A	2	3	2	7
D	5	3	9	17
U	8	14	7	29
Serial Amacrine	0	3	1	4
Area (μ^2)	520.5	585.7	585.7	1691.9

APPENDIX E

SUMMARY TABLES OF ANOVA

ON INCIDENCE COUNTS OF SYNAPSES

APPENDIX E - TABLE 1

Amacrine

Source	df	SS	MS	F	p
(btwn)	(19)	.04107045	.00216160		
A	3	.00265482	.00088494	.3686	ns
Ss/w.gr.	16	.03841563	.00240098		
(within)	(40)	.08107048	.00202676		
B	2	.06077385	.03038693	51.1935	<.01
BxA	6	.00130255	.00021709	0.3657	ns
BxSs/w.gr.	32	.01899408	.00059357		
Total	59				

APPENDIX E - TABLE 2

A/A

Source	df	SS	MS	F	p
(btwn)	(19)	.00454993			
A	3	.00007493	.00002164	.0772	ns
Ss/w.gr.	(16)	.00448500	.00028031		
Within	(40)	.00462301			
B	2	.00233571	.00116786	19.4093	<.01
BxA	6	.00036176	.00006029	1.0020	ns
BxSs/A	(32)	.00192554	.00006017		
Total	59	.00917294			

APPENDIX E - TABLE 3

A/B

Source	df	SS	MS	F	p
(btwn)	(19)	.00197492			
A	3	.00044898	.00014966	1.5692	ns
Ss/w.gr.	16	.00152594	.00009537		
(within)	(40)	.00275465			
B	2	.00034690	.00017345	2.7756	ns
BxA	6	.00040809	.00006802	1.0885	ns
BxSs/w.gr.	32	.00199966	.00006249		

APPENDIX E - TABLE 7

Bipolar

Source	df	SS	MS	F	p
(btwn)	(19)	.00096867			
A	3	.00023011	.00007670	1.6616	ns
Ss/w.gr.	16	.00073856	.00004616		
(within)	(40)	.00226049			
B	2	.00005247	.00002624	.4149	ns
BxA	6	.00018416	.00003069	.4852	ns
BxSs/w.gr.	32	.00202386	.00006325		

APPENDIX E - TABLE 6

Serial Amacrine

Source	df	SS	MS	F	p
(btwn)	(19)	.00046063			
A	3	.00006954	.00002318	.9483	ns
Ss/w.gr.	16	.00039109	.00002444		
(within)	(40)	.00053688			
B	2	.00020997	.00010499	11.4869	<.05
BxA	6	.00003434	.00000572	.6258	ns
BxSs/w.gr.	32	.00029257	.00000914		
Total	59				

APPENDIX E - TABLE 5

A/U

Source	df	SS	MS	F	p
(btwn)	(19)	.02631210			
A	3	.00677753	.00225918	1.8504	ns
Ss/w.gr.	16	.01953457	.00122091		
(within)	(40)	.03609599			
B	2	.02528317	.01264159	41.9443	<.01
BxA	6	.00116832	.00019472	.6461	ns
BxSs/w.gr.	32	.0096445	.00030139		
Total	59	.06240809			

APPENDIX E - TABLE 4

A/G

Source	df	SS	MS	F	p
(btwn)	(19)	.00397934			
A	3	.00032454	.00010818	.4736	ns
Ss/w.gr.	16	.00365480	.00022843		
(within)	(40)	.0051569			
B	2	.00158036	.00079018	10.6364	<.01
AxB	6	.00119932	.00019989	2.6907	<.05
BxSs/w.gr.	32	.00237722	.00007429		
Axb1	3	.00004928	.00001643	0.2212	ns
Axb2	3	.00016518	.00005506	0.7411	ns
Axb3	3	.00130939	.00043646	5.8751	<.05
Total	59	.00913624			

APPENDIX E - TABLE 8

B/A

Source	df	SS	MS	F	p
(btwn)	(19)	.00010715			
A	3	.00001891	.00000630	1.1423	ns
Ss/w.gr.	16	.00008824	.00000552		
(within)	(40)	.00016611			
B	2	.00002075	.00001038	2.7978	ns
BxA	6	.00002671	.00000445	1.1995	ns
BxSs/w.gr.	32	.00011865	.00000371		
Total	59	.00027326			

APPENDIX E - TABLE 9

B/D*

Source	df	SS	MS	F	p
(btwn)	(19)	.00030870			
A	3	.00001141	.00000380	.2045	ns
Ss/w.gr.	16	.00029729	.00001858		
(within)	(40)	.00032727			
B	2	.00001662	.00000831	1.1245	ns
BxA	6	.00007418	.00001236	1.6725	ns
BxSs/w.gr.	32	.00023647	.00000739		
Total	59	.00063597			

APPENDIX E - TABLE 10

B/U

Source	df	SS	MS	F	p
(btwn)	(19)	.00121802			
A	3	.00020959	.00006986	1.1084	ns
Ss/w.gr.	16	.00100843	.00006303		
(within)	(40)	.00168006			
B	2	.00010769	.00005385	1.1814	ns
BxA	6	.00011378	.00001896	.4160	ns
BxSs/w.gr.	32	.00145859	.00004558		
Total	59	.00289808			

APPENDIX F

COMPARISONS OF THE CHANGES IN SYNAPTIC INCIDENCES (EXPRESSED AS % OF CONTROLS) OF DIFFERENT TYPES OF CELLS IN THE IPL DUE TO LIGHT DAMAGE AND LIGHT DEPRIVATION. FIFKOVA'S LIGHT DAMAGED DATA IS FROM FIFKOVA (1973), HER LIGHT DEPRIVED DATA IS FROM FIFKOVA (1972b). * = NO STATISTICS REPORTED. CONTROL FOR THE PRESENT STUDY WAS THE UD GROUP.

APPENDIX F - TABLE 1

		Light Damaged (UB)	Light Damaged (SB)	Light Deprived (SN)
A/(Total)	Sosula & Glow			+ 137.0 <.001
	Fifkova	+ 25.4 *		+ 33.5 *
	Chernenko	+ 6.1 ns	+ 14.9 ns	+ 6.3 ns
A/A	Sosula & Glow			+ 120.1 <.001
	Fifkova	+ 13.6		+ 74.0 <.001
	Chernenko	+ 8.5	+ 2.4 ns	+ 14.2 ns
A/B	Sosula & Glow			+ 54.1 ns
	Fifkova	- 57.9		+ 19.0 <.001
	Chernenko	+ 14.0 ns	- 16.7 ns	+ 10.2 ns
A/G	Sosula & Glow			+ 245.7 <.001
	Fifkova	+ 154.5 *		- 1.5 ns
	Chernenko	+ 10.7 <.05 (Outer third)		+ 15.6 <.05 (Outer third)
A/U	Sosula & Glow			
	Fifkova			
	Chernenko	+ 0.1 ns	+ 36.1 ns	+ 0.3 ns
B/(Total)	Sosula & Glow			+ 49.2 ns
	Fifkova	- 11.4 *		- 18.7 <.02
	Chernenko	- 2.2 ns	- 8.2 ns	- 14.6 ns
B/A	Sosula & Glow			+ 50.0 ns
	Fifkova			
	Chernenko	- 11.4 ns	- 31.4 ns	- 20.0 ns
B/D	Sosula & Glow			+ 50.6 ns
	Fifkova			
	Chernenko	- 25.7 ns	- 17.1 ns	+ 14.3 ns
B/U	Sosula & Glow			
	Fifkova			
	Chernenko	- 3.8 ns	- 6.2 ns	- 23.3 ns