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Articles

Antagonistic effects of adrenalectomy and ether/surgical stress on light-induced photoreceptor damage

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Light-induced damage to retinal photoreceptors is influenced by the endocrine status of the animal during the period of exposure. Experimental manipulation of the pituitary gland and of prolactin levels has been shown to affect retinal damage in rats exposed to visible light. When rats are experimentally stressed, prolactin secretion from the pituitary gland occurs as does secretion of adrenocorticotropic hormone (ACTH), which stimulates the release of adrenal cortical hormones. Since prolactin appears to influence retinal damage and since stressed animals have increased serum levels of prolactin, a comparison of photoreceptor damage in animals in which the adrenal glands were removed or which had been experimentally stressed was undertaken in this study. Adrenalectomized rats had thicker outer nuclear layer (ONL) measurements than those found in sham-operated animals. Stressed rats had severely damaged retinas with cystic degeneration and significantly reduced ONL thickness measurements as compared to retinas of unstressed and adrenalectomized rats. Therefore hormones of the pituitary-adrenal system appear to be involved in the damage to the retina by light, and this response may be related to an interaction or synergism between the adrenal gland, stress, and prolactin secretion. (INVEST OPHTHALMOL VIS SCI 22:1-7, 1982.)

Key words: retina, photoreceptors, pituitary gland, prolactin, adrenocorticotropic hormone, stress, light damage

The severity of photically induced retinal photoreceptor damage is closely related to the hormonal state of the animal at the time of light exposure. Sexual immaturity, removal of the pituitary gland[1] and ovary,[2] and pinealectomy[3] tend to decrease the susceptibility of the retina to photic damage. Pituitary transplantation and extracts administered to hypophysectomized rats increase the susceptibility of the retina to light damage,[1] as does administration of estradiol[2] or prolactin.[1] Contrarily, follicle-stimulating (FSH) and luteinizing hormone (LH), progesterone, and alpha-melanocyte–stimulating hormone

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Table I. Stress and retinal damage in intact, adrenalectomized and hypophysectomized rats

Groups	*ONL (μm)*	*RT (μm)*
1. Sham-ADRENALEX (surgically stressed) (n = 9)	8.44 ± 0.97[A]	72.82 ± 2.18
2. ADRENALEX (surgically stressed) (n = 10)	13.69 ± 0.85	76.86 ± 1.76
3. Intact (unstressed) (n = 10)	15.71 ± 0.72	77.07 ± 1.59
4. Intact, unstressed (n = 6)	16.65 ± 1.18	78.91 ± 2.61
5. Intact, ether-stressed (n = 6)	8.25 ± 1.76[A]	66.98 ± 3.51
6. HYPEX, stressed (n = 6)	19.94 ± 1.82[B]	72.07 ± 4.67
7. HYPEX, ether-stressed (n = 6)	18.95 ± 0.65	73.63 ± 1.62
8. HYPEX, surgically stressed (n = 10)	17.15 ± 0.95	63.14 ± 1.20[C]

HYPEX = hypophysectomized rats; ADRENALEX = adrenalectomized rats.
Values are mean ± S.E.M.
p values; [A]$p < 0.001$ group 1 vs. 2 and 3, group 4 vs. 5; [B]$p < 0.05$ group 4 vs. 6; [C]$p < 0.05$ group 7 vs. 8.

(α-MSH)[4] do not appear to affect the susceptibility of the retina to light damage. Of these different hormonal conditions, removal of the pituitary gland and the augmentation of serum prolactin, from either endogenous or exogenous sources, appear to be the most effective procedures for modifying the severity of photoreceptor damage.

A close functional relationship apparently exists between the release of prolactin and stress, which is associated with increased adrenal function. Nicoll et al.[5] demonstrated that stresses (e.g., cold, intense light and heat) and physical restraint induced lactation in rats primed with estrogen and concluded that under these conditions prolactin and adrenocorticotropic hormone (ACTH) were released from the pituitary gland. Neill[6] demonstrated that ether anesthesia caused a substantial increase in serum prolactin levels in cycling female rats. The release of prolactin by ether stress and other forms of stress has been substantitated by other investigators[7–9]

The association of experimental conditions that elevate serum prolactin levels and increase susceptibility of the retina to photically induced damage and of stress-related adrenal cortical function led to the present study. Experiments have been designed to examine the susceptibility of retinal photoreceptors to light damage after bilateral removal of the adrenal glands and after ether and surgically induced stress.

Materials and methods

Female rats (Sprague-Dawley strain) were kept in a cyclic light environment (14 hr light:10 hr darkness; L:D) for at least 1 week prior to surgical removal of the adrenal glands under ether anesthesia. Bilateral adrenalectomy between the sixth and seventh weeks of age was accomplished by the dorsal approach. The sham operation was identical to adrenalectomy, except that the glands were only manipulated and not removed. Animals were given food and water ad libitum, but the drinking water of the adrenalectomized animals contained 0.9% sodium chloride. Groups of adrenalectomized and sham-operated rats were placed into polyethylene cages with wire tops and exposed to continuous fluorescent illumination (L:L) for 21 days. The light source had a spectral range of 397 to 732 nm, with a maximum at 590 nm, and an approximate intensity of 150 ft-c was measured with a Tektronix J16 digital photometer, with the illuminance probe directed toward the light source.

The pituitary glands were removed from 200-gram rats by the intra-aural approach, after which the animals were allowed to recuperate for at least 2 weeks prior to usage.

Two types of experimental stress were utilized. (1) *Ether stress.* Rats were anesthetized in an ether jar prepared by placing 5.0 ml of anesthetic ether on paper toweling; 1 to 2 min after addition of the ether, the animals were placed into the jar for 3 min and then removed. (2) *Surgical stress.* Other animals, while under ether anesthesia, were sham-operated bilaterally for adrenalectomy.

In the stress experiments, animals which had been ether-stressed or surgically stressed were placed into polyethylene cages along with unstressed control animals and exposed to 400 ft-c illuminance for 17.5 hr. Under this exposure regimen, approximately 40% of the total photoreceptor population of intact unstressed rats was damaged, as determined by outer nuclear layer (ONL) thickness measurements (Table I). By using the 17.5 hr exposure of high-intensity light instead of

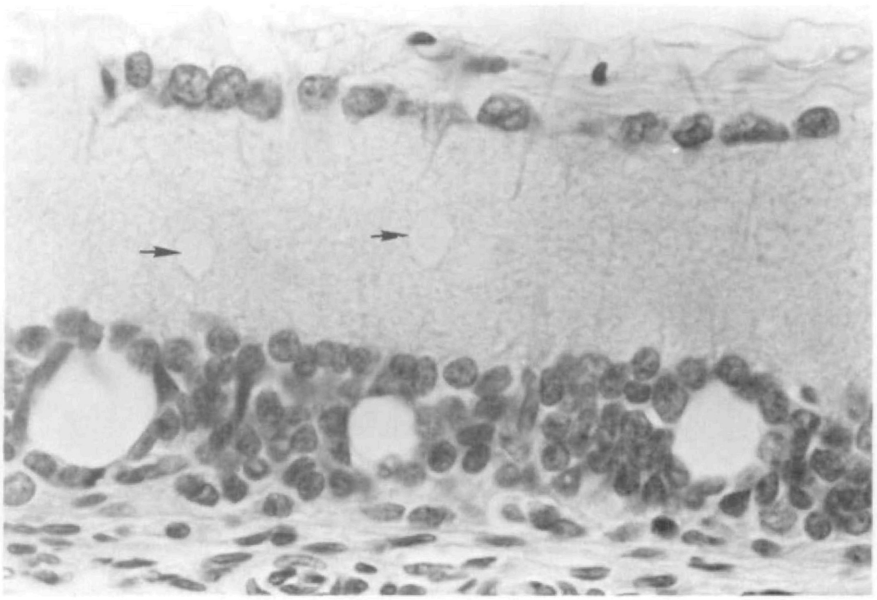

Fig. 1. Photomicrograph of a retina from a rat stressed by ether anesthesia prior to exposure to light. Note the absences of a distinct ONL (photoreceptor nuclei) and the presence of cysts in the inner nuclear and inner plexiform (arrows) layers. (×400.)

the 21-day exposure period of lower-intensity light, as described above, the degree of retinal damage per unit time was more easily regulated and sequenced in time in relation to the period of stress. Only two animals, one control and one stressed, were placed into each cage for light exposure. After exposure, the animals were returned to a special animal room with cyclic lighting (L: D) for 2 weeks prior to autopsy, since a postexposure period is necessary for phagocytic removal of damaged photoreceptor cells from the retina.[3]

The intensity of the illuminative source and temperature of the environment were carefully regulated as described. However, in these and earlier experiments, the effect of light damage on ONL thickness varied during different months of the year and among animals (all Sprague-Dawley descendants) from different vendors. Therefore control groups were included in every experiment, and statistical comparisons were not made among experiments.

At the end of each exposure period, the animals were anesthetized with ether and exsanguinated, and the eyes were enucleated. The superior surface of each eye was marked with a felt-tip pen for future orientation of the tissue sections. The eyes were fixed for 4 hr in Bouin's solution, dehydrated in an alcohol and benzene series, and embedded in paraffin. Tissue blocks were sectioned at 7 μm thickness on the anterior-posterior axis, and sections of the central retina, including the optic nerve, were stained with Harris' hematoxylin and eosin. The following measurements were made on each retina with an ocular micrometer at 400× magnification: (1) ONL thickness, measured from the outer limiting membrane inward to include all photoreceptor nuclei, and (2) retinal thickness (RT), the distance from the ONL to the inner margin of the ganglion cell layer. The two measurements were taken at 12 different loci around the circumference of each retinal section, beginning at the periphery. Loci were separated by a distance of approximately 540 μm.

At autopsy the animals were carefully examined to validate the absence of adrenal tissue.

Statistical comparisons between the control and each of the experimental groups were based on the Student t test.

Results

Routine histopathologic and morphometric examinations of each retina indicated that by 2 to 3 weeks after exposure to light, the damaged photoreceptors had been phagocytized, and only occasional macrophages were observed at this period.

Significant destruction of photoreceptors, as determined by observations and measurements of the ONL thickness, occurred in control and experimental retinas in all exposed groups of animals, but the degree of

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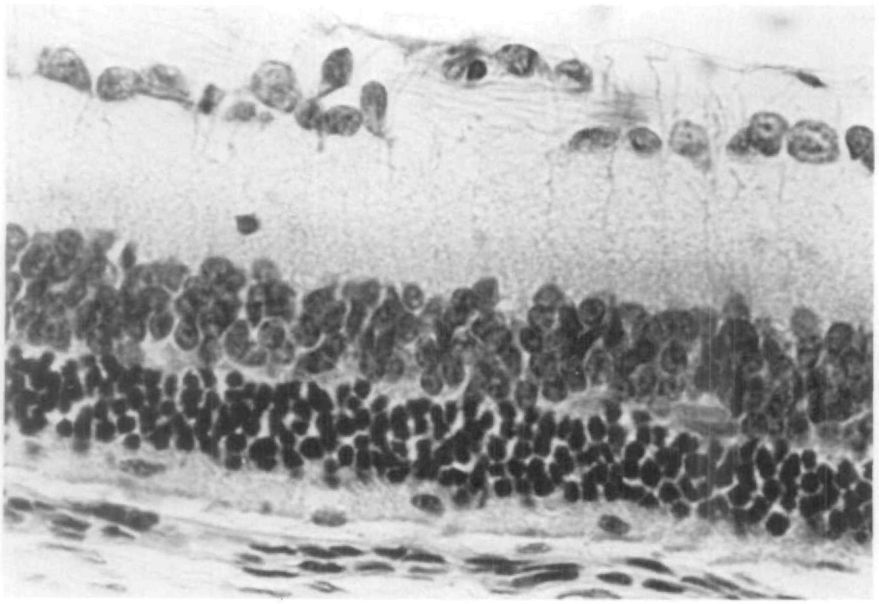

Fig. 2. Photomicrograph of a retina from a control rat, which was unstressed prior to exposure to light, illustrating a region of the retina comparable to that of the stressed rat in Fig. 1. A distinct ONL consisting of approximately 50% of the original photoreceptor nuclei remains. Cystic degeneration was not evident in these retinas, which showed reduced light-induced damage as compared to that of stressed rats. (×400.)

severity was significantly modified by the experimental procedures. In general, photoreceptor destruction was found throughout the peripheral and central retina, but the most severe destruction occurred in the superior hemisphere at approximately 2100 to 2600 μm from the ora serrata. At this locus, the bipolar layer (inner nuclear layer) was juxtapositioned to the pigment epithelium and choroid coat, and intact rod and cones were absent. Nuclei of photoreceptors, especially cone nuclei, were scattered in this zone but did not compose a single complete layer in severe damage.

A distinctive structural abnormality of exposed retinas of the stressed, as compared to unstressed animals, was a significant cystoid degeneration (Figs. 1 and 2), which consisted of large vacuolated, nonvascular spaces lying within the inner nuclear layer and sometimes extending throughout it. Small cysts were also seen in the ONL, inner plexiform layer, and ganglion cell layer of the retinas of stressed animals. There was no other apparent modification or damage to neurons of the inner nuclear or ganglion cell layers, but morphometric studies were not made of these cell populations.

Adrenalectomized animals. The thickness (mean ± S.E.M.) of the ONL of the intact (unoperated) animals (n = 10) was 10.21 ± 0.50 μm, and that of the sham-operated rats (n = 10) was 10.57 ± 0.28; the difference between the two groups was statistically insignificant. However, the ONL thickness of the adrenalectomized groups (n = 10) was 13.79 ± 0.96 μm, a statistically significant difference ($p < 0.01$) of 26.0% and 23.4% vs. the intact and sham-operated groups. A graphic display of the morphometric data (Fig. 3) from the different regions of the retinas indicated that at all loci the intact and sham-operated retinas were similarly damaged. On the other hand, the ONL measurements at all regions of adrenalectomized rat retinas were distinctly different from the control groups. The area of most severe receptor destruction in all animal groups occurred at locus 4 (Fig. 3) on the superior retina.

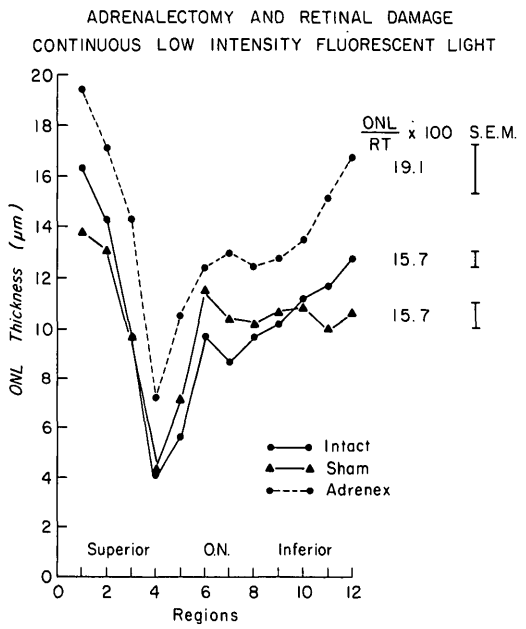

Fig. 3. ONL thickness in retinas of intact, sham-operated and adrenalectomized rats subjected to 3 weeks of continuous fluorescent illumination. In the abscissa, the peripheral retina is at the left and right of the optic nerve *(O.N.)*.

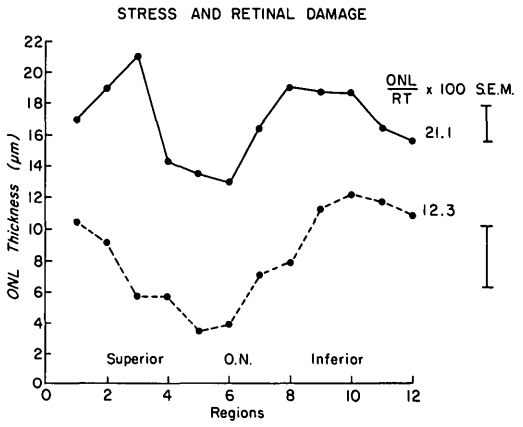

Fig. 4. ONL thickness in retinas of stressed rats *(broken line)* and unstressed rats *(solid line)* after exposure to a damaging photoperiod. Note in Figs. 3 and 4 that the regions having the most severe damage lie adjacent to the optic nerve *(O.N.)* on the superior hemisphere.

When the experiment was repeated 1 month later, the ONL measurements (mean ± S.E.M.) were as follows: sham-operated control group, 8.45 ± 0.97 μm (n = 10); adrenalectomized group, 13.69 ± 0.85 (n = 10). The difference of the means was highly significant ($p < 0.001$; 38.3%).

Stressed groups. In the first stress experiment, which involved a comparison of retinal damage in unstressed, unoperated, in sham-adrenalectomized (ether anesthesia and surgical stress), and in adrenalectomized (ether anesthesia and surgical stress) rats, the animals were exposed to the 17.5 hr photoperiod immediately after surgery and then were returned to the cyclic photoperiod for a 2-week stabilization period. ONL thicknesses were 15.71 ± 0.72 μm for the unstressed group (n = 10) and 8.44 ± 0.97 for the stressed group (n = 8). The difference in the means was statistically significant ($p < 0.001$; 46.3%). The ONL thickness of surgically stressed animals (group 2, Table I) in which the adrenal gland had been removed prior to light exposure did not differ significantly from that of the intact, unoperated group (group 3) but was statistically different from the ONL thickness of the sham-operated, surgically stressed group (group 1).

Subsequently, a second comparison of retinal damage under conditions similar to those in the first experiment was made in groups of unstressed, unoperated and of ether stressed, unoperated rats. In addition, retinal damage was examined in the following three groups of animals in which the pituitary gland was removed: (1) unstressed, unoperated, (2) ether stress, unoperated, (3) surgical stress in sham-operated rats. A 2-week recovery period elapsed between the time of hypophysectomy (HYPEX) and exposure to damaging photoperiod so that surgical stress would not be a variable in these animals.

A statistically significant difference occurred between the means of the ONL thicknesses of intact vs. ether-stressed groups ($p < 0.001$; 50.5%), and in the entire RT measurements of the two groups ($p < 0.05$; 15.1%) (Table I, Fig. 4).

Table I also shows the results of ONL and entire RT measurements of the three HYPEX groups. The mean of the ONL thickness of unstressed animals did not differ

significantly from that of the ether-stressed or surgically stressed groups. However, the entire RT of the surgically stressed group was significantly reduced as compared to that of the ether-stressed group. Means of the ONL thickness of retinas of unstressed HYPEX rats were statistically greater than those of unstressed, intact animals, but the difference in the means of the RT measurements of the two groups was not significant.

Discussion

In these experiments related to the effects of visible light and hormonal status of the animal on photoreceptor damage, the intensity of the light source and length of the exposure period were selected to permit an evaluation of the experimental variables rather than to destroy all photoreceptor cells in the retina. Exposure of the animals to the visible fluorescent light source resulted in severe damage to the retina and a resulting reduction in the thickness of the ONL, as previously reported by many authors (for review see ref. 3). Interestingly, the superior retina was significantly more severely damaged than the inferior portion, an observation which is in agreement with the results of Rapp and Williams.[10] Another observation from the present study that corroborates previous results[1] was the occurrence of a thicker ONL in hypophysectomized as compared to intact animals, which could be interpreted as indicating that the pituitary gland regulates either the severity of light damage to photoreceptors or possibly the rate of phagocytosis of damaged cells.

The pituitary-adrenal system apparently influences the damaging effect of visible light on rods and cones of the rodent eye. The retinas of rats in which the adrenals had been removed and therefore in which the serum levels of adrenal hormones had been greatly reduced had significantly less damage than the retinas of sham-operated control groups.

On the other hand, animals which were stressed by ether exposure or by ether-surgery possessed retinas with much greater damage than their unstressed control groups, as indicated by reduced ONL thickness measurements. In addition, the acutely damaged retinas of stressed rats presented a severe cystic degeneration involving all layers of their structure, but this histopathologic feature was absent in retinas of unstressed animals of this experimental series. Interestingly, Noell et al.[11] associated cystic degeneration in their study with severely damaged retinas after prolonged exposure to light at high body temperature.

Stress is a functional state characterized by increased, but transient, serum levels of prolactin and ACTH. Previous studies have indicated the existence of an inverse relationship between serum prolactin levels and the thickness of the ONL.[3] Therefore the results of the present experiments involving stress-activated prolactin release substantiate the earlier report. However, the retinal pathology was much more severe in the stressed animals than in unstressed rats, an observation that might indicate an interaction of prolactin with the pituitary-adrenal system. As discussed above, removal of the adrenal glands, i.e., reduction of serum levels of adrenal hormones, in unstressed groups ameliorated the damaging effects of light on photoreceptors. Also, light-induced photoreceptor damage was significantly more severe in surgically stressed intact animals than in surgically stressed adrenalectomized rats (Table I), an observation that appears to implicate adrenal hormones and photoreceptor damage. The interrelation between the pituitary-adrenal system and prolactin is being investigated to determine whether ACTH or an adrenal cortical hormone is involved in retinal photoreceptor damage.

REFERENCES

1. O'Steen WK and Kraeer SL: Effects of hypophysectomy, pituitary gland homogenates and transplants, and prolactin on photoreceptor destruction. INVEST OPHTHALMOL VIS SCI 16:940, 1977.
2. O'Steen WK: Ovarian steroid effects on light-induced retinal photoreceptor damage. Exp Eye Res 25:361, 1977.
3. O'Steen WK: Hormonal influences on retinal photodamage. *In* The Effects of Constant Light on Visual Processes, Williams TP and Baker BN, editors. New York, 1980, Plenum Press, p. 29.
4. O'Steen WK and Kastin AJ: Relationship of

melanocyte-stimulating hormone to photoreceptor damage. Peptides 1:19, 1980.
5. Nicoll CS, Talwaker PK, and Meites JM: Initiation of lactation in rats by nonspecific stresses. Am J Physiol 198:1103, 1960.
6. Neill JD: Effect of stress on serum prolactin and luteinizing hormone levels during the estrous cycle in the rat. Endocrinology 87:1192, 1970.
7. Wakabayashi H, Arimura A, and Schally AV: Effect of pentobarbital and ether on serum prolactin levels in rats. Proc Soc Exp Biol Med 37:1181, 1974.
8. Euker JE, Meites J, and Reigle GD: Effect of acute stress on serum LH and prolactin in intact, castrate and dexamethasone treated rats. Endocrinology 96:85, 1975.
9. Rossier J, French E, Rivier C, Shibasaki T, Guillemin R, and Bloom FE: Stress-induced release of prolactin: blockage by dexamethasone and naloxone may indicate β-endorphin mediation. Proc Natl Acad Sci USA 77:666, 1980.
10. Rapp LM and Williams TP: A parametric study of retinal light damage in albino and pigmented rats. *In* The Effects of Constant Light on Visual Processes, Williams TP and Baker BN, editors. New York, 1980, Plenum Press, p. 135.
11. Noell WK, Walker VS, Kang BS, and Berman S: Retinal damage by light in rats. INVEST OPHTHALMOL 5:450, 1966.