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Neuronal Damage in the Rat Retina After Chronic Stress

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Long-term exposure to escapable foot shock has been used to determine if chronic stress influences neuronal cell death in the retina of albino and pigmented rats. Histopathologic and morphometric approaches analyzed changes in photoreceptors and neurons of the bipolar and ganglion cell layers of the retina. Albino Fischer rats when exposed to chronic stress for 4–8 h daily for 1 week to 6 months, developed severe retinal damage, as compared to unstressed control retinas, with reduction in photoreceptor and bipolar neurons, particularly in the superior central retina. The damage was observed in male and female rats, but males appeared to be more susceptible to the influence of stress than female animals. Ganglion cells were unaffected. Photoreceptor destruction did not occur in Long-Evans pigmented rats under identical experimental conditions. The results suggest that: (1) input of the sensory stimulus, light, to the retina of stressed rats augmented neuronal damage and might be required for its initiation; and (2) hormones and/or neurotransmitters associated with long-term chronic stress might be related to increased neuronal cell death in the mammalian retina.

INTRODUCTION

Both acute and chronic stresses act as stimuli for the release of hormones, such as ACTH and prolactin, from the pituitary gland[12,13]. In addition to altering the functional state of the animal, hormones have been demonstrated to influence the structure of neurons and neuroglial cells and neuronal cell death in the central nervous system[3,6–9,11,26]. The neural retina is derived developmentally from the diencephalon, and its structural, functional and biochemical features closely resemble those of classical subdivisions of the CNS. Therefore, neuronal populations in the retina may be expected to respond to hormones in a manner similar to those in the CNS.

The hormonal status of an animal influences retinal photoreceptor cell death induced by exposure to intense or extended periods of visible light[14–18]. Retinas of young rats[1,20] and hypophysectomized rats[16,23] are protected from photic damage as compared to those of adult and intact animals. However, the implantation of a pituitary gland into hypophysectomized rats increases the severity of photoreceptor damage[23]. The presence of certain hormones, such as prolactin and estrogen[17,23], causes increased retinal damage when given to hypophysectomized or ovariectomized animals, respectively, while other hormones, such as α-MSH[22], LH, FSH and progesterone have little or no effect on this phenomenon[23].

Acute ether or surgical stress, when initiated prior to photic exposure, increases retinal cell death. When the adrenal gland, the target organ for pituitary ACTH and the source of corticosteroids, is removed, photoreceptor cell death is reduced[21]. These observations suggest that hormones of the pituitary gland and/or the adrenal gland, in some manner, influence the severity of neuronal cell death in the mammalian retina.

The present study was undertaken to determine if chronic stress influences neuronal cell death in the retina of albino and pigmented rats. Histopathologic and morphometric methods were utilized to analyze changes in the photoreceptors and neurons of the bipolar and ganglion cell layers.

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MATERIALS AND METHODS

The model for applying chronic stress consisted of exposing rats to a situation that was an aversive, but not damaging, shock-escape conditioning. Rats were placed in a large plexiglass box that was subdivided into four compartments, each of which could hold 6–10 animals. The floor of the chamber was an electrified grid wired to provide current to either the front or back half of all compartments. The electrified side was reversed at random intervals with a relay click just preceding the reversal. The animals received a mild foot-shock which resulted in their moving to the opposite side of the compartment. The voltage delivered was set by the investigator to apply the smallest shock to the animals and yet retain its aversiveness. The current reversal intervals were programmed to occur randomly from 30 to 300 s with animals being exposed to currents of less than 1.0 mA. Therefore, the animals could limit exposure to the shock by responding quickly to the signal. All animals learned to escape (100% criterion) within 2 s after 1–2 sessions in the chambers, and therefore the amount of shock to each animal was approximately equal. The rats usually remained motionless until the relay click and subsequent shock occurred.

All animals were kept in wire mesh cages in the animal room except during the 4 or 8 h period of daily chronic stress. They were stressed daily from Monday through Friday, and not on weekends. The 4 h periods of stress occurred during the morning hours of each day.

Water and rat chow were provided ad libitum during the darkness feeding period and daylight hours, except during the daily period of stress. Body weights of experimental animals were reduced during the first 2 weeks of stress, but, thereafter, increased at a rate comparable to that of unstressed control groups. Animal care and treatment followed the guidelines of and were approved by the University Animal Care Committee and are accredited by AAALAC.

Experiment I

Adult male Fischer rats (10–12 weeks old) were divided into six experimental groups (n = 12 each), which were exposed to chronic stress for either 4 or 8 h each day for either 1, 2, or 4 weeks (5-day weeks). Six control groups (n = 6 each) were maintained in the same manner, but were not stressed. Prior to and during the stress period, the rats were maintained on food and water ad libitum and on a cyclic photoperiod (14 h light: 10 h darkness, 14:10 LD). The light intensity in the animal colony cages (front to back) was 6–2 ft-cd, as measured with a Tektronix-J16 digital photometer with the illuminance probe located at animal eye level and directed towards the light source. Animals aggregated and slept at the back of the cage. The fluorescent lighting in the laboratory where stress was applied was measured at 105 ft-cd.

Experiment II

Adult male and female Fischer rats (6 months old) were divided into control (n = 9 each) and experimental (n = 12 each) groups. The experimental group was chronically stressed for 4 h each day for 8 weeks under the same environmental conditions as those in Experiment I.

Experiment III

Adult female Fischer rats (6 months old) were divided into control (n = 9 each) and experimental (n = 9 each) groups. The experimental group was stressed chronically for 4 h daily for 6 months under the same environmental conditions as those in Experiment I.

Experiment IV

Adult male Fischer rats (6 months old) were divided into control (n = 5 each) and experimental (n = 5 each) groups. The experimental group was stressed chronically for 4 h daily for 4 months, but the light intensity in the stress laboratory was reduced from 105 ft-cd used in the first three experiments to 77 ft-cd for Experiment IV.

Experiment V

Adult male Long-Evans pigmented rats (3 months old) were divided into unstressed control (n = 10 each) and stressed experimental (n = 10 each) groups. The experimental groups were stressed chronically for 4 h each day for one month under the same experimental conditions as those in Experiment I.

The eyes were removed at autopsy, and the superior surface was marked with an indelible felt-tip pen

for future orientation during sectioning. They were fixed for 5 h in Bouin's solution, dehydrated in an alcohol and xylene series, and embedded in paraffin. Tissue blocks were sectioned at 7 μm on the anterior-posterior axis, and sections of the central retina including the optic nerve were stained with Harris' hematoxylin and eosin. Slide labels were masked with paper tape prior to histopathologic and morphometric evaluation, so that the source (stressed or unstressed groups) of the tissue was unknown to the evaluator. The following measurements were made on each retina with an ocular micrometer at 400 × magnification: (1) outer nuclear layer thickness (ONL), measured from the outer limiting membrane inward to include all photoreceptor nuclei; (2) retinal thickness (RT), the distance from the outer limiting membrane to the inner margin of the ganglion cell layer; and (3) inner nuclear layer thickness (INL), the distance from the outer to inner margins of the bipolar, amacrine, and horizontal neurons (in Experiments I and IV only). The three measurements were taken at 12 different loci around the circumference of each retinal section, beginning at the periphery (Fig. 1). Loci were separated by a distance of approximately 450 μm. Ganglion cell neurons were counted in the superior retina of each animal along a total distance of 1275 μm, beginning at locus 4 and extending to the optic papilla in Experiments I and IV only. Also, neurons of the bipolar layer were counted along a total distance of 200 μm at locus 5 in retinas of Experiments I and IV.

Statistical significance was determined by comparing data from two groups of animals in each experiment by Student's *t*-test, or when among groups, by Duncan's multiple range test.

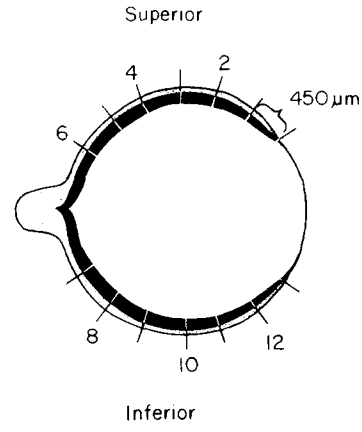

Fig. 1. Diagram of a sagittal section of the rat eye (cornea, right; optic nerve, left), demonstrating the 12 loci of the superior and inferior retina from which retinal, outer nuclear layer, and inner nuclear layer thicknesses were measured. Distance between loci = 450 μm. Ganglion cells were counted along a distance of 1275 μm from locus 4 to the optic nerve-optic papilla. Neurons in the bipolar layer were counted along a distance of 200 μm at locus 5.

RESULTS

General histopathology

Examination of the retinas of chronically stressed rats in Exp. I indicated a noticeable and significant reduction in the thickness of the outer nuclear layer and a disruption of the outer segments of the photoreceptors. Structural modifications were greatest in groups exposed to stress for 8 h each day for 4 weeks. The superior hemisphere of the eye had more severe retinal damage than the inferior half (Figs. 2, 5 and 6). The ONL did not appear to be affected as severely in the peripheral retina as in the central part, but the bipolar neuronal population appeared to be reduced peripherally in the stressed groups. However, the nuclei of the bipolar neurons (INL) and photoreceptors (ONL) were scattered among each other and were not organized into distinct layers, especially in the peripheral retina of long-term stressed groups. In general, the thinnest region of the retina was in the superior central retina at a distance of 1800–2700 μm from the ora serrata, but restricted focal lesions (e.g. 135 μm in length) were also observed in the superior and inferior retina at approximately 630 μm from the ora serrata. At these foci, the entire retinal thickness was reduced noticeably, and the nuclear strata were disorganized with photoreceptor nuclei scattered among bipolar nuclei. Vacuolar spaces, indicative of cystic degeneration of the retina, were present in the inner nuclear and inner plexiform layers of rats stressed for 8 h each day for 4 weeks. In Experiment IV when the room light intensity was reduced from 105 to 77 ft-cd, the photoreceptor layer of the entire retina was reduced in thickness in peripheral and central areas, but the extreme superior retinal damage seen at locus 5 in other experiments was not observed (compare Figs. 2, 3 with 4).

Morphometric analyses indicated that chronic stress significantly reduced the thicknesses of the

TABLE I

Effects of chronic stress on the thickness of the outer nuclear layer (ONL) and entire retina (RT) of the rat eye

ONL and RT are expressed as the mean (μm) ± S.E.M. Animals were stressed for either 4 or 8 h/day for either 1, 2 or 4 weeks (5-day weeks). All animals were maintained in a cyclic photoperiod (14 h light:10 h darkness). Control groups (C), n = 6 rats each; experimental groups (E), n = 12 rats each.

Group no.	*Treatment regimen (h/day × week(s))*	*ONL*		*RT*	
		C	*E*	*C*	*E*
1	4 × 1	33.54 ± 0.50	32.41 ± 0.27*	99.01 ± 1.88	96.62 ± 1.04
2	8 × 1	32.95 ± 0.32	28.72 ± 0.40**	96.55 ± 1.42	90.92 ± 1.08***
3	4 × 2	32.79 ± 0.53	32.29 ± 0.34	98.09 ± 1.71	95.57 ± 1.25
4	8 × 2	33.27 ± 0.43	30.80 ± 0.49***	99.63 ± 1.27	93.68 ± 1.84*
5	4 × 4	32.05 ± 0.30	30.32 ± 0.37***	95.49 ± 1.06	88.88 ± 0.98**
6	8 × 4	32.74 ± 0.50	27.71 ± 0.35**	96.09 ± 1.87	83.50 ± 1.19**

* $P < 0.05$, significant difference between experimental and its control group.
** $P < 0.001$.
*** $P < 0.01$.

ONL and of the entire retina (RT). For Experiment I, however, chronic stress for only 1 or 2 weeks for 4 h each day reduced the ONL at borderline ($P < 0.05$) significance, or not at all, and retinal thickness was unchanged (Table I), as compared to that of controls. Statistically significant reductions in ONL and RT occurred after 4 h of stress for 4 weeks and after 8 h of stress for 1, 2 or 4 weeks. The greatest difference between control and experimental groups in Experiment I was recorded after 8 h of daily stress for 4 weeks. Chronic stress for 8 h daily for 4 weeks did not change the INL thickness or the number of ganglion cells in the superior retina as compared to unstressed control data (Table II). A statistically significant reduction in the number of bipolar neurons occurred in chronically stressed rats (Table II).

TABLE II

Effects of chronic stress on thickness of the inner nuclear layer (INL, μm ± S.E.M.) and on bipolar neuron and ganglion cell counts

Animals of Experiment I were stressed for 4 weeks at 8 h per day, while rats of Experiment IV were stressed for 4 months at 4 h per day. Numbers in parentheses indicate number of animals per group. Bipolar neurons (BPN) and ganglion cells (GC) were counted on a 200 μm and 1275 μm section of the central superior retina, respectively.

	INL	*BPN*	*GC*
Exp. I			
Unstressed (6)	20.0±0.6	148.3±4.9	125.9±3.6
Stressed (12)	19.9±0.4	133.3±3.3*	127.0±3.1
Exp. IV			
Unstressed (5)	21.2±0.6	158.6±5.2***	115.6±8.5***
Stressed (5)	17.3±0.4**	127.2±3.7**	117.2±6.1

* $P < 0.02$, unstressed vs stressed.
** $P < 0.001$, Exp. IV, unstressed vs stressed.
*** P NS, Exp. I vs Exp. IV, unstressed controls.

The histopathology of the stressed male and female rats in Experiment II resembled that in Experiment I; that is, reduction in the number of rows of photoreceptors, poorly defined borders of the ONL, and an area of most severe damage at locus 5 on the superior retina. Retinas from unstressed animals had well-defined layers of nuclei composed of from 9 to 12 rows of ONL nuclei in the central retina.

Morphometric analyses (Fig. 2) of the retinas in Experiment II indicated that the superior ONL was thinner than the inferior ONL in both male and female stressed rats, as compared to their control groups; the measurements verified the histologic observation that the most severe damage was at locus 5, although significant reductions in ONL thickness occurred at loci 3 and 4. The mean values of the male control and stressed ONL measurements were statistically different, while those of the female groups were not. The difference between ONL measurements of control animals in Experiment I (32.74 ± 0.50 μm, group 6) and Experiment II (29.90 ± 0.30 μm, males) was statistically significant ($P < 0.05$).

In Experiment III, the histopathology of the retina did not vary from that found in Experiments I and II (Figs. 5 and 6). However, the ONL of the control animals (Fig. 3) was significantly thinner than it was in the previous experiments. Morphometric analyses demonstrated that the area of most severe damage

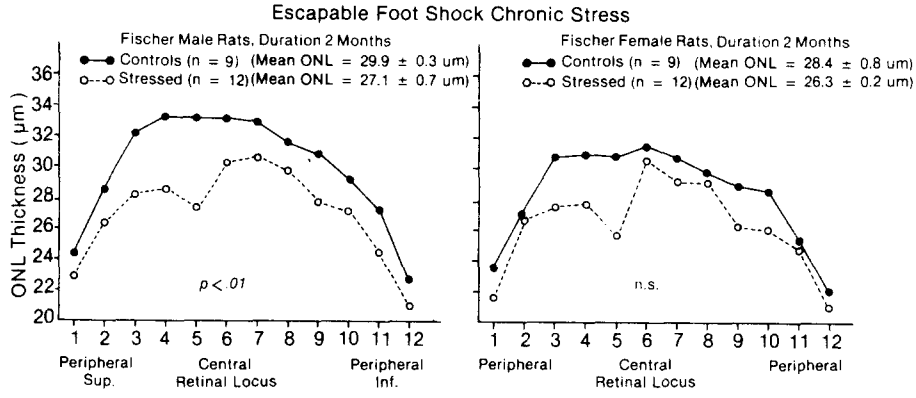

Fig. 2. Outer nuclear layer (ONL) thickness of rats exposed to chronic escapable foot-shock stress for 4 h daily for 2 months. Measurements (mean ± S.E.M.) were made at 12 different loci of the superior (sup.) and inferior (inf.) peripheral and central retina. Note the severe reduction of photoreceptor nuclei (ONL) at loci 3, 4 and 5 in the superior retina of stressed male and female rats, with significant reduction occurring in male animals only.

to, or reduction of, the photoreceptors was at locus 5, but indicated significant loss of photoreceptors at other loci (Fig. 3) in the retinas of stressed rats. The difference in the means of retinas from female control rats and rats stressed for 6 months was highly significant statistically ($P < 0.001$).

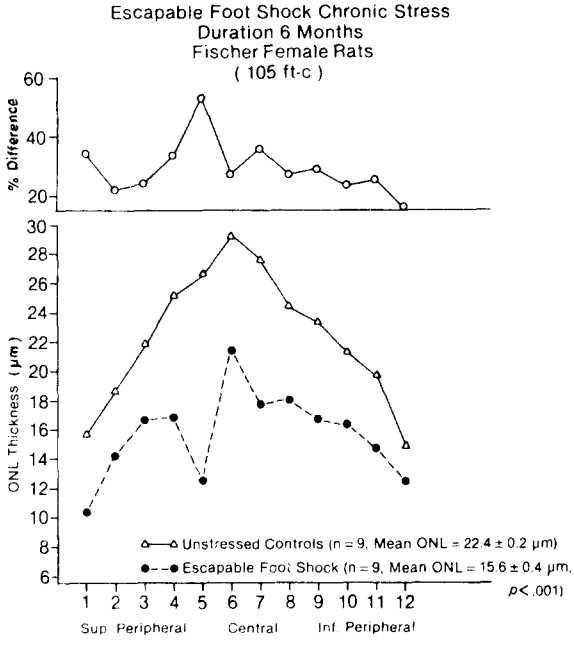

Fig. 3. Outer nuclear layer (ONL) thickness of female rats exposed to chronic stress for 4 h daily for 6 months, demonstrating a significant ONL reduction throughout the retina. The most severe loss of photoreceptor nuclei occurred at loci 4 and 5 (superior retina) in stressed rats. Means ± S.E.M. and percent difference between control and experimental measurements at each loci are provided.

In Experiment IV, environmental conditions of the animals were identical to those in the first three experiments, except that the intensity of the fluorescent lighting during the period of stress was reduced from 105 ft-cd to 77 ft-cd. Two obvious changes in the histopathology were observed, as compared to previous experiments: (1) the loss of photoreceptors at locus 5 on the retina was not as severe proportionally to that in other loci; and (2) the peripheral retina (superior and inferior) showed an obvious reduction in the ONL, an observation substantiated by the morphometric analyses (Fig. 4). The measurements of the ONL, when placed on a graph (Fig. 4), result in a dome-shaped configuration for the retinas of both the control and stressed groups. A similar configuration was found to represent the thickness of the inner nuclear layer (INL) around the semicircular extent of the retinas of the stressed and unstressed groups (Fig. 7). Rats that had been stressed daily for 4 months at lower light intensities than in previous experiments had significantly smaller mean INL measurements than those in the unstressed control group (Table II). The superior retinal INL was significantly thinner than that of the inferior sector ($P < 0.001$), except at the periphery of the retina (loci 1 and 2); the zones of greatest reduction in INL thickness occurred at loci 4 (25.6%) and 5 (28.1%) in stressed rats, as compared to control measurements ($P < 0.001$). A highly significant reduction in the number of bipolar neurons occurred in the stressed group (Table II), as was observed in Experiment I. The number of neurons in the ganglion cell layer of

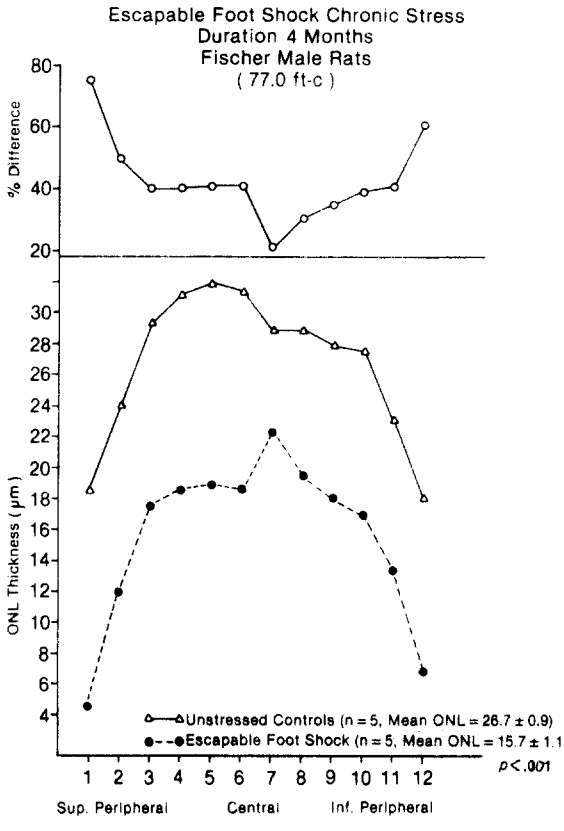

Fig. 4. ONL thickness (means ± S.E.M.) of male rats exposed to chronic stress for 4 h daily for 4 months. Animals were kept in 77 ft-cd of fluorescent illuminance, in contrast to 105 ft-cd illuminance used in Experiments I, II and III. Stressed rats showed a significant reduction of ONL thickness throughout the retina, with greatest damage occurring in loci 3, 4 and 5 of the superior retina.

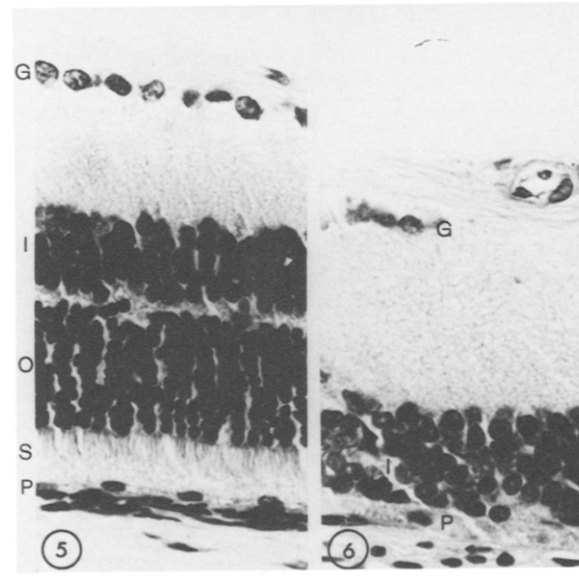

Fig. 5. Photomicrograph of the central retina (locus 5) of an unstressed rat illustrating the normal integrity of neuronal layers in a 12-month-old Fischer rat. G, ganglion cell layer; I, inner nuclear layer (bipolar neurons); O, outer nuclear layer (photoreceptor nuclei); S, inner and outer segments of photoreceptors; P, pigment epithelium. × 667.

Fig. 6. Photomicrograph of the central retina (locus 5) of a 6-month-old rat that was stressed chronically for 6 months (final age 12 months, Experiment III). Note the reduction in overall retinal thickness, destruction and absence of photoreceptor nuclei and inner and outer segments, and the close approximation of the inner nuclear layer (I) to the pigment epithelium (P). × 667.

stressed rats did not differ significantly from that of unstressed animals in each experiment (Table II). The apparent reduction in ganglion cell number between the control retinas of Experiments I (125.9 ± 3.6) and IV (115.6 ± 8.5) was statistically insignificant. When the ganglion cell numbers of all animals in Experiment I (mean 126.6 ± 2.4, n = 18) were compared with those of Experiment IV (mean 116.4 ± 5.0, n = 10), the difference between the two groups was of borderline significance ($P < 0.05$).

In Experiment V the environmental conditions of the pigmented rats were identical to those in the first three experiments, and the rats were chronically stressed for 4 h daily for one month. Retinas of stressed pigmented rats did not differ histologically from those of unstressed control animals. The thickness of neither the ONL (38.6 ± 0.5 μm) nor the RT (117.8 ± 1.5 μm) of the unstressed control rats (n = 10) was statistically significant from that of the ONL (38.7 ± 0.4 μm) and RT (117.8 ± 1.5 μm) of the chronically stressed animals (n = 10). However, a significant difference occurred between the ONL thickness of the control Fischer albino (Group 5, Table I), and the control Long-Evans pigmented rats, and between the entire retinal thickness (Table I, $P < 0.01$) of control groups of the two strains, both of which were from experiments of one month's duration. Differences between the entire retinal thicknesses of the stressed albino and pigmented groups were highly significant ($P < 0.001$), as were the differences between the ONL thickness of the two experimental groups (Group 5, Table I, $P < 0.001$).

DISCUSSION

Albino Fischer rats, when exposed to chronic es-

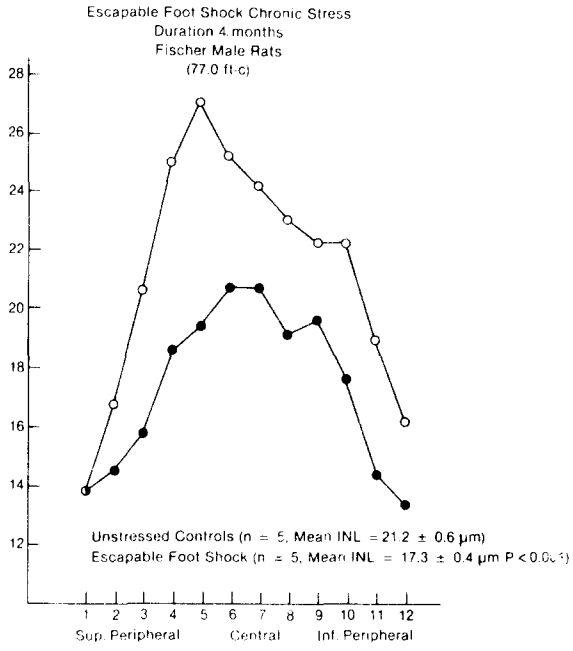

Fig. 7. The inner nuclear layer (INL) thickness was reduced significantly by exposure to chronic stress for 4 h/day for 4 months at a reduced level of cyclic light intensity (77 ft-cd), as compared to that of Experiments I, II and III. The greatest reduction occurred in the central retina, especially at loci 4, 5 and 6. Refer to Table II for mean INL ± S.E.M. measurements, as compared to cell counts of bipolar layer neurons. ○, unstressed controls; ●, escapable foot shock.

capable foot-shock stress for periods of from one week to six months, developed a more severe degree of retinal damage than was found in control rats which were unstressed. The stressed rats were exposed to either 4 or 8 h of escapable foot-shock per day during the light portion of the cyclic photoperiod.

Both histopathologic and morphometric criteria were utilized to evaluate retinal damage in control and stressed animals. The most obvious histopathologic changes included a reduction in the number of rows of photoreceptor nuclei in the outer nuclear layer (ONL), the occurrence of cystic spaces among the bipolar neurons of the inner nuclear layer (INL) and in the inner plexiform layer, and in most instances (Experiments I, II and III), a more extensive loss of photoreceptor nuclei in the superior central retina than in the inferior sectors. The number of neurons in the bipolar layer appeared to be reduced after 1 and 4 months of chronic stress, and these neurons often were scattered among photoreceptor nuclei. Ganglion cell layer neurons appeared to be unaffected by chronic stress for periods up to 4 months.

Morphometric analyses substantiated the histopathologic observations on the loci of most severe damage. When the data for each of the 12 loci around the retina were plotted, the severity of the damage at superior loci 3, 4 and 5 became obvious. A greater loss of photoreceptors in the superior hemisphere of the retina, as contrasted with the inferior half, has been described for retinas after light damage[24], particularly after extended periods of daily exposure, continuous long-term exposure, or exposure to intense illuminances[21,22]. Therefore, the pattern of stress-induced retinal damage in Experiments I, II and III resembles closely that of photically induced damage even though in these experiments, the stressed (and control) rats were kept in cyclic light, and the control rat retinas did not show noticeable or measurable loss of photoreceptor nuclei.

The observation that the absolute reduction in thickness of the entire retina exceeded the absolute reduction in ONL thickness (Exp. I, Table I) indicates that RT reduction involves a factor in addition to the loss of photoreceptor nuclei (ONL). However, the most obvious histopathologic change was the loss of photoreceptor nuclei. In Experiment IV (Table II and Fig. 7), the bipolar layer (INL) was thinner, and bipolar neurons were reduced in number, especially obvious in the superior half of retinas of stressed rats. Therefore, the reduction in INL thickness (Experiment IV), and in INL cell count, also contributed to the overall reduction in retinal thickness. Dendritic and synaptic processes of retinal neurons (plexiform layers) were included in the RT measurement, and damage to these components might be related to the reduction in RT. However, these retinal layers were not measured individually in morphometric analyses in this study.

Two experimental conditions may account for the reduction in the number of bipolar neurons in Experiments I and IV: (1) chronic stress influences, which may be mediated through hormones or neurotransmitters; and (2) transneuronal degeneration following the death of photoreceptors. Chronic stress exposure caused extensive photoreceptor damage, as compared to the control rats, and the stressed groups had significantly fewer bipolar neurons than the controls (Table II). Even though a significant reduction in the number of bipolar neurons occurred in Experiments I and IV, the thickness of the INL was not af-

fected except in Experiment IV, after 4 months of chronic stress exposure. Therefore, the loss of bipolar neurons in these animals was not reflected accurately by the INL thickness measurements, as reported by others[27].

Although ganglion cell populations were unaffected by chronic stress in Experiments I and IV, there was a reduction of approximately 8% in the total ganglion cell population between the two groups. This reduction in ganglion cells may be related to the age of the rats in Experiment I (5 months) as compared to those in Experiment IV (10 months). Shinowara et al.[27] observed a 7.7% decrease in bipolar neurons between the ages of 3 and 12 months in Fischer rats, but did not count ganglion cells in their study; however, the thickness of the ganglion cell layer and inner plexiform layer, which was measured, was unchanged during that period.

When the illuminance was reduced from 105 ft-cd to 77 ft-cd during the 4-h stress period each day, the damage to loci 3, 4 and 5 was less severe than that recorded at the higher intensity. However, the difference in ONL thickness between the control and stressed groups at the lower intensity was statistically highly significant ($P < 0.001$, Fig. 4). Perhaps, the influence of the higher intensity illuminance on retinal damage was overriding the effect of stress in Experiments I, II and III, and the individual consequence of stress on these neurons is better demonstrated at the lower intensity of sensory input. It is interesting that the percent difference between each locus in the retinas of unstressed and stressed animals kept at the lower illuminance (Experiment IV, Fig. 4) is greater in the peripheral retina (60–75%) than in other loci, a pattern of retinal damage which has been observed with aging in the Fischer rat[5,27].

The possibility that light stimulus to the photoreceptor is necessary, or at least synergistic, for the occurrence of the destructive influence of chronic stress must be considered in speculating on mechanisms for stress-induced neuronal cell death in the retina. Additional evidence for the essentiality of the photic stimulus for stress-induced neuronal damage is provided by the observation that, under the same experimental environment, photoreceptor destruction did not occur in pigmented rats. Apparently, in pigmented rats, chronic stress exposure alone did not exacerbate neuronal cell death in the absence of an illuminance comparable to that reaching the surface of the albino rat retina. Light-induced photoreceptor damage can occur in retinas of pigmented rats, but it depends on a higher intensity of illuminance, longer exposure period, or increased environmental or body temperature[14,15,29]. Dilation of the pupil of pigmented eyes with atropine also enhances retinal damage[14,15].

The statistically significant difference between the retinas of male control rats of Experiment I and those of Experiments II and IV may be related to the age of the animals at autopsy, which was 5, 8 and 10 months, respectively. The female control rats of Experiment II and III were 8 and 12 months old at autopsy, and the latter group had a thinner ONL than the former one. These observations substantiate the changes in thickness in aging Fischer rat retinas reported by Shinowara et al.[27].

The mechanism for stress-induced damage to the retina observed in these experiments cannot be ascertained from the present data. The possibility of an endocrine influence is suggested by the observation that male rats were more susceptible to chronic stress than female rats in Experiment II under identical conditions. However, when female rats of the same age were stressed for 6 months (Exp. IV), rather than for two months (Exp. II), a significant reduction occurred in the ONL thickness in comparison to the control groups. Therefore, the data indicate that males are more susceptible than females to stress-induced retinal damage, but that the retina of female rats can be significantly damaged if chronic escapable foot-shock stress is continued for longer periods. The gender difference in susceptibility to retinal damage and the amelioration of the effect by removal of certain glands under different experimental conditions[16–19], strongly suggest that a hormonal mechanism may be involved directly or indirectly with neuronal cell death and that hormones associated with stress (e.g. ACTH, prolactin, corticosterone, norepinephrine) should be investigated.

In conclusion, exposure of rats to chronic escapable foot-shock stress significantly enhanced cell death of photoreceptors and neurons in the bipolar layer of the retina. Male rats appeared to be more susceptible to the stress than did females. Since the influence of chronic stress was not observed in pigmented rats under identical experimental conditions,

we concluded that input of the sensory stimulus, light, into the eye augmented the neuronal damage and might be required for its initiation. The results of this study indicated that the neuronal components of the retina might act as a model system for the analysis of the influence of chronic stress on the central nervous system.

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