Visual Dysfunction in Aged Fischer 344 Rats

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ABSTRACT. To investigate age-related changes in visual function in rats, male and female Fischer 344 (F344) rats at 30 months of age were examined electrophysiologically and histopathologically. The selection rate for the dark area in a T-shaped test box was 80% in aged rats, and the ability of light-dark discrimination was definitely depressed. Electroretinogram (ERG) was non-recordable in 25 out of the 28 eye balls examined, and amplitudes of the ERG a- and b-waves were markedly depressed in the remaining three eye balls. Histopathologic examination of the retina revealed marked atrophy of photoreceptor cells on the outer nuclear and photoreceptor layers; the change was less extensive in the retina of eye balls in which ERG was recordable. Immunohistochemically, increased reactivity to anti-glial fibrillary acid protein serum was observed in the retina of the aged animals. These results evidenced that the number of photoreceptor cells is decreased in aged F344 rats, resulting in the reduced reactivity to light and the depressed light-dark discrimination.—KEY WORDS: aging, Fischer 344 rat, visual function.

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It is important to differentiate treatment-related changes from spontaneously occurring age-related changes for accurate assessment in toxicity studies. Fischer 344 (F344) rats are widely used for long-term toxicity studies including carcinogenicity studies. Although there are several reports on age-related morphological changes in the retina in F344 rats [3, 4, 6–8], little is known about the visual function in aged rats of this strain.

In this study we examined the change of visual function in aged F344 rats using behavioral and electrophysiological methods in addition to histopathologic examination of the retina.

MATERIALS AND METHODS

Animals: Fourteen F344/Jcl rats aging 30 months old were served as aged groups and twenty F344/Jcl rats aging 4 months old as young control groups in the present study.

These rats, at an age of 4 weeks, were purchased from CLEA Japan Inc. (Shiga, Japan) and maintained individually in wire-mesh bottomed stainless steel cages. Each cage was allocated randomly to a position on a shelf in a room of which environments were maintained constant, i.e., 12/12 hr light-dark cycle (light on at 8 a.m. and light off at 8 p.m.), a temperature of 23±3°C and a relative humidity of 55±10%. The animals had free access to tap water and a laboratory rat diet (CE-2, CLEA Japan Inc.). The animals were reared without any treatment until 4 or 30 months of age.

Light-dark discrimination test: The light-dark discrimination was estimated on the basis of negative phototaxis by using a T-shaped light-dark test box. The selection rate for the dark area, calculated as the average of 4 trials, was measured as a possible parameter of visual function.

Ophthalmoscopic examination: The ocular fundus in both eyes of each animal was examined with a fundus camera (Kowa RC-2 model 621, Kowa Co., Ltd., Tokyo, Japan). The examination was performed 5–10 min after instillation of a mydriatic (Midrin®-P, Santen Pharmaceutical Co., Ltd., Osaka, Japan).

Electroretinogram (ERG): In order to record the ERG, rats were anesthetized with ketamine hydrochloride (Ketalar®, Sankyo Co., Ltd., Tokyo, Japan; 100 mg/kg, i.m.) and their pupils were dilated with a mydriatic (Midrin®-P). The recording of ERG was repeated several times for both eyes using a contact lens type electrode (Kyoto Contact Lens, Kyoto, Japan) and a conducting solution (Scopisol®, Senju Pharmaceutical Co., Ltd., Osaka, Japan). Prior to the recording, the animal was placed in a dark room for 30 min. A photostimulator (MSP-2R, Nihon Koden, Tokyo, Japan) and a xenon lamp were used to generate a 100-μsec light flash stimulus at 10 sec intervals. The ERGs were amplified with an amplifier (Type 1205D, San-ei Instrument Co., Ltd., Tokyo, Japan; time constant = 0.1 sec) and 10 successive responses were averaged by a signal processor (7T07A, San-ei Instrument Co., Ltd.; sweep time = 200 msec). After the processing, the averaged wave form was photographed.

Pathological procedures: Each rat was exsanguinated under ether anesthesia after the ophthalmologic examination. All the eyeballs were fixed for about three minutes in 4% phosphate-buffered glutaraldehyde solution, post-fixed in 5 % neutral buffered formalin, embedded in paraffin. These samples were sectioned at a thickness of 4 μm and stained with hematoxylin and eosin (HE). For immunohistochemistry, selected paraffin-embedded sections were also examined using a streptavidin-biotin-peroxidase (HISTOFINE SAB-PO kit, Nichirei Corporation, Tokyo, Japan) method with trypsin digestion for anti-bovine S-100 protein, anti-glial fibrillary acid protein (GFAP) and anti-neuron-specific enolase (NSE) polyclonal rabbit antibodies (Nichirei Corporation). Sections of the brains of normal male F344/DuCrj rats (104 weeks of

age) were used as controls.

RESULTS

Light-dark discrimination test: As shown in Table 1, the average selection rate for the dark area in 4 trials was 80% in aged rats (males: 71%, females: 89%) compared to 100% in young rats.

Ophthalmoscopic examination: No abnormalities were seen in the ocular fundus in aged or young rats.

ERG: No electrical activity in response to photostimulation was recognized in 25 out of the 28 eyes in aged rats even if the flash intensity was much high as 2.0 joules (Fig. 1-c). In the remaining 3 eyes, amplitudes of the a- and b-waves were markedly decreased (Fig. 1-b) as compared with those in the young controls (Fig. 1-a).

Histopathology: In the three eyes of which ERGs were recordable with decreased amplitudes, the thickness of the outer nuclear and photoreceptor layers was reduced. For instance, the outer nuclear layer in the three eyes was only about 6 nuclei thick (Fig. 2-b) compared to approximately 12 nuclei thick in young rats (Fig. 2-a).

Table 1. Rate of selection of the dark area (%)

Age (Months)	Sex	Number of animals	Trials 1st	2nd	3rd	4th	Average
4	Male	10	100	100	100	100	100
	Female	10	100	100	100	100	100
		20	100	100	100	100	100
30	Male	7	71	71	57	86	71
	Female	7	100	86	86	86	89
		14	86	79	71	86	80

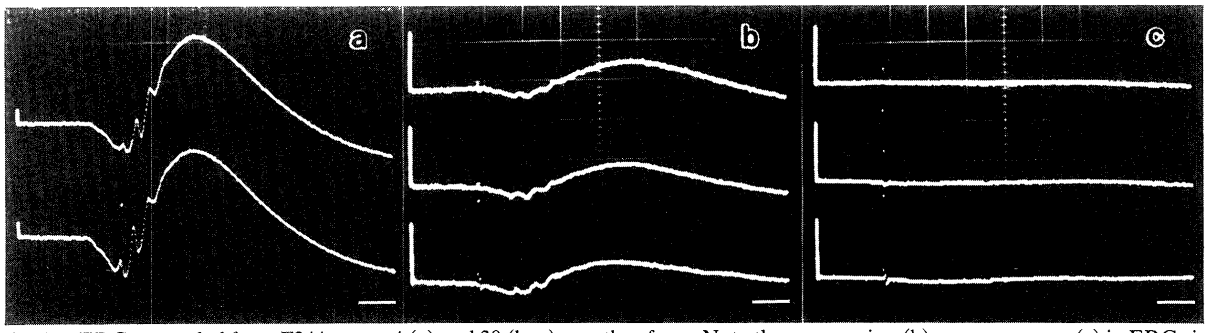

Fig. 1. ERGs recorded from F344 rats at 4 (a) and 30 (b, c) months of age. Note the suppression (b) or no response (c) in ERGs in aged rats. The flash intensities were, from the top, 0.3, 0.6 and 2.0 (b and c only) joules. A time marker shows 20 msec. The vertical bar on each trace indicates 50 μV.

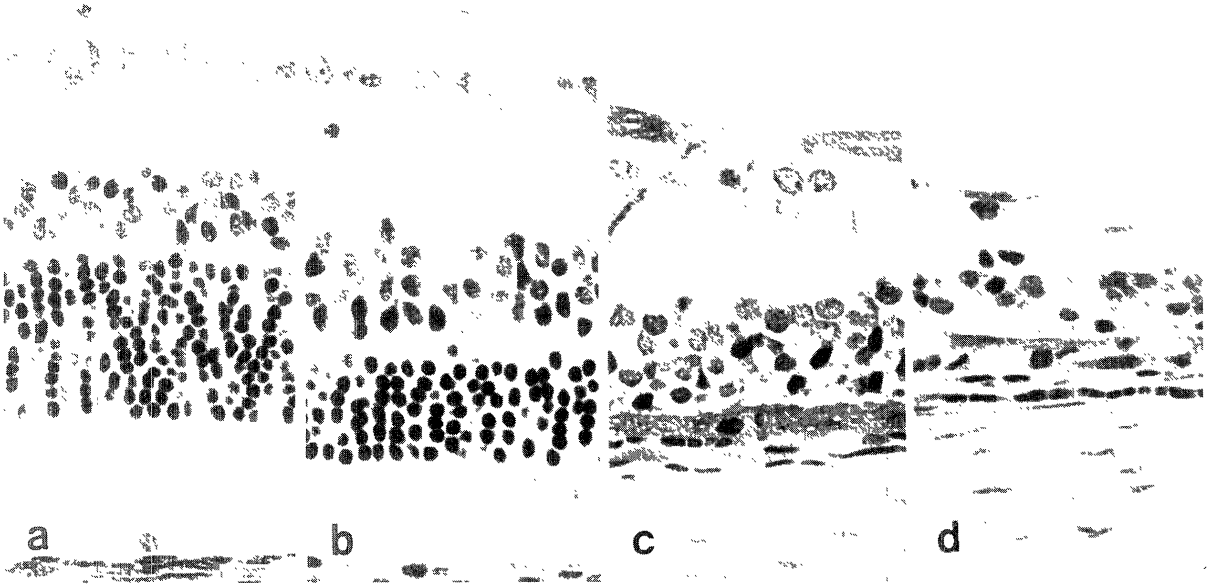

Fig. 2. Photomicrographs of the retina from F344 rats at 4 (a) and 30 (b, c, d) months of age. HE stain. × 440. a: The retina is well developed and the outer nuclear layer is about 12 cells thick. b: All retinal layers are present, but the outer nuclear layer is only 6 cells thick. The photoreceptor layer is also reduced. c: The photoreceptor cells are completely lost, while the pigment epithelium and the inner retinal layers are normal. d: The degeneration has extended to the inner retinal layers, and the normal structure is lost.

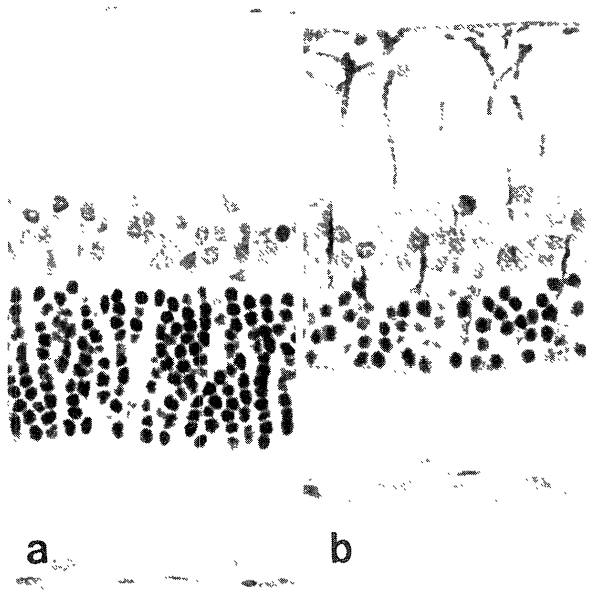

Fig. 3. Photomicrographs of the retina from F344 rats at 4 (a) and 30 (b) months of age. GFAP stain. × 440. Strong GFAP immunoreactivity is seen in aged rats (b).

In the eyes of which ERGs were non-recordable, the decrease in the thickness of the outer nuclear and photoreceptor layers was more prominent, and photoreceptor cells were almost completely lost. However, the pigment epithelium and the inner retinal layers were intact (Fig. 2-c). In several cases, the degeneration extended to the inner retinal layers, and the normal structure of the retina was lost completely (Fig. 2-d).

Immunohistochemically, there were no differences between aged rats and young rats in NSE or S-100 immunoreactivity in the retina. Strongly GFAP-positive fibers were observed in the retinal layers except for the photoreceptor layer in aged rats, probably in the Müller cells (Fig. 3-b), whereas these were not recognized in young rats (Fig. 3-a).

DISCUSSION

In the present study, visual function in aged rats was behaviorally and electrophysiologically investigated in comparison with that in young rats.

In the light-dark discrimination test the average selection rate for the dark area decreased to 80 % in aged rats, which no young rats selected the light area. A light-dark discrimination test using negative phototaxis is one of simplified methods for estimating the visual function in rats. A similar method is also used as a behavioral teratology screening test for visual abnormalities [1]. The results of the present light-dark discrimination test indicate that normal visual function in the aged rats was markedly deteriorated, but not abolished since the selection rate for the dark area in completely blind rats might be theoretically 50%. Furthermore, no responsibility in ERG to the photostimulation in most of aging rats indicated that the reactivity of the retina to light in these animals was considerably depressed. This depression should be closely associated with the lowered light-dark discrimination ability in aged rats.

Histopathological changes in the eyes in aged rats have been characterized by the selective loss of photoreceptor cells in the retina [2–4 , 6–8 , 10]. The pathologic findings observed in this study were basically the same as those reported previously. Such pathological changes are considered to be advanced as follows [3 ,4]. At an early stage (Grade 1), the outer nuclear and photoreceptor layers are selectively reduced. At a more advanced stage (Grade 2), an almost complete loss of photoreceptor cells occurs, but the inner retinal layers and the pigment epithelium remain normal. At a late stage (Grade 3), the degeneration extends to the entire retina, and the normal retinal structure is lost.

In the present study, ERG response was markedly depressed but still present in the eyes with Grade 1 lesions. In the eyes with more advanced lesions (Grade 2 or 3), the ERG response was completely abolished. The size of the ERG response seems to be related to the number of remaining photoreceptor cells. The present results obtained from the ERG recordings suggest that the reduced reactivity to light in the retina in aged rats is at least a consequence of a decrease in the number of photoreceptor cells.

The lack of changes in retinal NSE immunoreactivity suggests that the ganglion cells and nerve fibers in the retina remain normal in aged rats. Despite the number of Müller cells is conceived to be less changed from the results of S-100 immunoreactivity, strongly positive GFAP immunoreactivity was widely observed in the retina in the aged rats. It has been reported that the Müller cells which are negative for GFAP in the normal state become positive for GFAP after retinal damage [9]. The strong GFAP-expression observed in the present study may indicate that the Müller cells react to the retinal degeneration as glial cells in the retina in aged rats.

Although light-induced retinal damage in albino rats is well known [5], it is assumed that the retinal degeneration observed in the present study is an age-related change, since this change has also been reported to occur in retina exposed to dim light [3]. In pigmented rats the age-related cell loss in the retina was also reported [2]; however, such change is observed to a minor extent as compared with that in the present study. Retinal degeneration in albino rats associated with aging may be exaggerated by exposure to light or stress [3, 4, 6, 7].

These results suggest that visual function in the aged rats was depressed by a decrease in the number of photoreceptor cells followed by a reduction in the reactivity to light in the retina. The results also indicated that age-related visual dysfunction should be taken into account in long-term studies in rats.

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