Age-Related Retinal Function Changes in Albino and Pigmented Rats

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PURPOSE. To investigate the effect of old age (3 vs. 18 months) on the retinal function of albino (Sprague-Dawley [SD]) and pigmented (Long-Evans [LE]) rats.

METHODS. Electroretinograms (ERG) were recorded in both albino (SD; 3 months old n = 16, 18 months old n = 16) and pigmented (LE; 3 months n = 16, 18 months n = 5) rats. Data are analyzed for photoreceptor, ON-bipolar, and retinal ganglion cell (RGC) amplitudes as well as photoreceptor and ON-bipolar cell sensitivities.

RESULTS. In the pigmented strain, senescence results in decreased photoreceptor output, but ON-bipolar and retinal ganglion cell amplitudes were preserved, due to a relative increase in ON-bipolar cell sensitivity. In the albino rats, although ageing decreased both photoreceptor and ON-bipolar cell amplitudes, increased photoreceptor sensitivity produced a relative sparing of retinal ganglion cell amplitude.

CONCLUSIONS. Both strains show evidence of retinal plasticity with senescence, albeit at different retinal levels. The exact mechanisms underlying sensitivity changes require further investigation. Nevertheless, given the findings of previous human studies, pigmented rats appear to be a more appropriate model for human ageing. Future work using animals to study the effect of ageing need careful consideration in strain selection. (Invest Ophthalmol Vis Sci. 2011;52:8891–8899) DOI:10.1167/iovs.11-7602

Aging has long been associated with a decline in sensory function, as observed in animal1–4 and human4–6 studies. Age-related visual changes in humans include reduced visual acuity,6 visual field sensitivity,7,8 contrast sensitivity,9–11 and delayed dark adaptation.12 These functional changes are associated with structural changes in the ageing eye, such as crystalline lens opacification,13 blood vessel calcification,14,15 and an increased oxidative stress.16 Furthermore, there is evidence of a decline in the number of neurons with age in human eyes, including losses of photoreceptors,17,18 rod bipolar cells,19 retinal ganglion cells,20 and retinal pigmented epithelial cells,21,22 that might explain some aspects of functional decline.

Many retinal disease models have been developed in mice and rats. Often these diseases are induced in relatively young rodents, which may be appropriate in some cases but is not ideal for studies of age-related neurodegenerative conditions. It is clear that age is a robust risk factor for neurodegenerative conditions such as glaucoma and age-related maculopathy. Thus, using older animals may provide a more appropriate backdrop over which to consider disease-specific risk factors. It is worth noting that age-related neuronal losses have been observed in rat eyes.23–26 Despite these anatomic findings, few studies have considered whether retinal function in rodents changes with age. Moreover, it is not clear whether different cell classes in the eye show greater functional loss with age.

One way to make such a comparison is to simultaneously assess the function of various retinal cell classes via the electroretinogram (ERG).27 Human studies have shown that, with age, there is a decrease in ERG a- and b-wave amplitudes28–30 that reflects changes in outer and middle retinal function. Similar findings have also been reported in rats.31 Although distal retinal function has received much attention, age-related changes in responses arising from the proximal retina have not been extensively studied. However, Sannita et al.32 found that the high-frequency oscillatory potentials of the ERG, thought to arise from amacrine cells, increase during early life (5 years old) and show a downward trend after the fifth decade in humans. In rats, OP amplitudes have been shown to decrease from approximately 17 weeks of age on.28 As the retina is a serial processing system, it is not clear how much of this inner retinal decline reflects upstream expression of photoreceptor or bipolar cell changes.

To our knowledge, no study has directly compared age-related functional changes across the photoreceptors, bipolar cells, and ganglion cells in albino and pigmented rats. As such, the following study was performed to consider the effect of normal ageing on various ERG waveforms arising from the distal and proximal retina (reflecting various subclasses of retinal neurons) in two commonly used species: albino Sprague-Dawley and pigmented Long-Evans rats.

MATERIALS AND METHODS

Animals

All experimental procedures abided with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and approval was obtained from our faculty’s Animal Ethics Committee. Male Long-Evans (LE) and Sprague-Dawley (SD) rats were housed with a 12:12-hour light–dark cycle (maximum, 50 lux) with food and water available ad libitum. In this study, four separate cohorts were assayed: 3-month-old SD (n = 16), 18-month-old SD (n = 16), and 18-month-old LE (n = 5) rats. All young animals (3 months old) were housed in our facility for a minimum of 1 month, and the old animals (18 months old) for a minimum of 6 months before experimentation.

Electroretinography

ERGs were recorded with custom-made chlorided silver electrodes, with the active one placed on the cornea and a ring-shaped reference placed around the sclera. The ground electrode (F-E2-30; Grass Tele-
factor. West Warwick, RI) was inserted into the tail subcutaneously. Eye drops containing carmelllose sodium (Celluviss 10 mg/mL; Allergan, Irvine, CA) were used to improve electrical contact and to maintain corneal hydration.²⁵

The ERG stimulus was delivered by either seven 8-W LEDs (bright flash) or a single 0.1-W LED (dim flash; Luxeon, Philips Lumileds Lighting Co., San Jose, CA), both housed in a Ganzfeld integrating sphere (Photometric Solutions International Pty Ltd., Huntindale, VIC, Australia). This system was calibrated before the experiment by using an integrating photometer (model IL-1700; International Light Technologies Inc, Peabody, MA) with a scotopic filter in place. After calibration, a series of gradually increasing luminous energies (−6.53 to 2.07 log cd · s · m⁻²) was chosen to provide a protocol spanning the dynamic range of the rat ERG. A twin-flash (500-ms interstimulus interval) was used at the brightest energy to identify the cone response.³¹

Before ERG recordings, animals were anesthetized via intramuscular injection of ketamine/xylazine (60 and 5 mg/kg; Troy Laboratories Pty Ltd., Smithfield, NSW, Australia) followed by topical application of propanetacaine (0.5%; Alcon Laboratories, Frenchs Forest, NSW, Australia). Mydriasis was achieved by application of single drops of tropicamide (0.5%, Alcon Laboratories) and phenylephrine hydrochloride (2.5%; Chauvin Pharmaceuticals Ltd., Kingston-upon-Thames, UK). After application of these preparations, the rodent’s head was placed in the plane of the Ganzfeld sphere and exposed to the luminous energy series described above, for ERG recording. Body temperature was controlled to 37°C with a circulating warm water pad throughout the experiment.

**ERG Analysis**

**Photoreceptor Response.** The leading edge of the scotopic a-wave was modeled by a delayed Gaussian based on the model of Lamb and Pugh³² and as formulated by Hood and Birch⁵⁵

\[
P_3(i, t) = Rm_{rP}[1 - \exp(-i \cdot S \cdot (t - t_0)^2)], t > t_0 \tag{1}\]

In equation 1, the leading edge of the P₃ (in microvolts) response for a given luminous energy (t, log cd · s · m⁻²) is expressed as a function of time (t in seconds), by its saturated amplitude (RmᵣP) and sensitivity (S, log m² · cd⁻¹ · s⁻¹) after a brief delay (t₀ in seconds). The delay (t₀) takes into account latencies in the phototransduction cascade, but also reflects a lag in the recording equipment. Although the delay may not be fixed for all luminous energies,³⁴ for simplicity, we chose to fix t₀ at 3.49 ms for all data. This value was determined by fitting the model to the average waveform. RmᵣP and S were optimized by minimizing the sum-of-square merit function (Solver module in Excel; Microsoft, Redmond, WA) across an ensemble of the top three luminous energies (1.78, 1.95, 2.07 log cd · s · m⁻²). For all models, the Q-statistic goodness of fit³⁵ was greater than 0.80 and can thus be regarded as an excellent fit.

**Rod and Cone ON-Bipolar Cell Response.** To isolate the bipolar cell component (P2), we subtracted the modeled P₃ from the raw ERG. The P₂ energy-amplitude function shows two phases that are likely to reflect contributions from rod and cone pathways.²⁵,²⁶ To quantify the entire relationship, the P₂ energy-amplitude response function was modeled with two saturating hyperbolic functions: one for rod and one for cone-mediated responses. Intensities where the rod response dominates were identified as those that were not significantly larger than the rod response isolated by subtracting the cone response from the raw ERG (twin flash paradigm).³¹ The first hyperbolic function was fit up to −1.42 log cd · s · m⁻²; whereas the second hyperbolic function was fit to luminous energies greater than this level. Each saturating hyperbolic function is given by equation 2:

\[
V(t) = V_{max} \frac{I}{I + k} \tag{2}\]

where the P₂ response amplitude (V, μV) is defined as a function of luminous energy (I, log cd · s · m⁻²), Vₘₐₓ (in microvolts) is the saturated amplitude of the response, and k is the flash energy for semisaturation (log cd · s · m⁻²). Vₘₐₓ and k were optimized to minimize the sum-of-square merit function (Solver module, Excel; Microsoft).

**Scotopic Threshold Response (STR).** In rats, the positive (pSTR) is thought to reflect responses involving ganglion cells, whereas the negative (nSTR) may also receive contributions from other inner retina neurons, such as amacrine cells.³⁷ The STR amplitude was measured from baseline at a fixed time of 130 ms (pSTR) and 240 ms (nSTR) across the dim luminous energies (−6.2 to −5.26 log cd · s · m⁻²). Because of the small amplitude of both pSTR and nSTR, data were averaged across these luminous energies to give a single pSTR and nSTR value for each animal.

**Identifying the Locus of Loss**

As ERG components reflect retinal processes that occur in series, any changes to the output of a particular cell class can influence the response of the downstream retinal cells. For example, in a serial process, a large phototransduction Rinₚ₂ results in a proportionally larger ON-bipolar cell–driven Vₘₐₓ. To quantify this effect, each ERG component was expressed as an amplitude ratio of the preceding ERG response, a process first described by Benolken et al.³⁸ More specifically, for each young animal, Vₘₐₓ/Rinₚ₂, pSTR/Vₘₐₓ, and nSTR/Vₘₐₓ were obtained and compared against the old cohort for each strain. Rod-isolated Vₘₐₓ was used in this analysis, as all the other parameters are rod dominated.

A ratio analysis effectively normalizes changes in input from the upstream component, with departures from values established in the young eyes providing information as to which component is most affected by age. This analysis was performed only between ages, not between strains. Our logic was that, as the ERG components are generated by neurons wired in series, they will yield a fixed-amplitude ratio in the presence of a generalized amplitude reduction, as demonstrated by Perlman.³⁹

**Statistics**

Group data are summarized as the average ± SEM. In comparing the effect of age and strain, two-way ANOVA was used with the Bonferroni post hoc adjustment at each age. In comparisons of amplitude response as a function of luminous energy, a repeated-measures two-way ANOVA (dependent variables: age, luminous energy) with the Bonferroni post hoc test was used. To compare the ratios (obtained by normalizing downstream ERG responses to its upstream component) between the two ages of the same strain, the Mann-Whitney U test (nonparametric) was used.

**RESULTS**

The raw waveform stack in Figure 1 shows that ERG amplitude decreased with age in both strains. Figures 1A and 1E show group average ERG waveforms for the LE rats at selected luminous energies. The thick traces represent group average waveforms from the 18-month-old compared with the 3-month-old rats (thin traces). Figure 1E shows that the pSTR amplitude did not change with age (slight delay in older eyes); however, the nSTR appeared to be smaller in the older group. At brighter luminous energies (Fig. 1A), the a- and b-waves appeared slightly delayed and smaller in the older LE rats.

In contrast, the ageing effect on ERGs appeared different in the albino rats (Figs. 1C, 1G). At dim luminous energies (Fig. 1G), the pSTR and nSTR appear to show little change in the younger albino rats (Figs. 1C, 1G). At dim luminous energies (Fig. 1G), the pSTR and nSTR appear to show little change in the older group.
1A vs. Fig. 1C), whereas the inner retinal nSTR was paradoxically less affected by age in the SD rats (Fig. 1E vs. Fig. 1G).

The cone responses (isolated from the twin-flash paradigm) collected at 2.07 log cd⋅s⋅m⁻² in the two age groups of the LE and SD rats are considered in Figures 1F and 1H. Figure 1F shows that in the LE rats, the maximum cone response was slightly decreased in the older eyes (thick trace). This amplitude reduction was more severe in the SD waveforms (Fig. 1H).

In regard to how changes in the a-wave might influence the downstream b-wave, Figure 1B shows waveforms from the young (thin trace) and old LE (thick trace) rats normalized to their own group average a-wave maximum. The same normalization is shown for the SD rats in Figure 1D. This shows that the leading edge of the a-wave was slower in the older LE rats when compared to their younger counterparts (inset); however, the contrary was found in the SD rats (inset). Moreover, for a matched a-wave, the b-wave of the older LE rats returned similar amplitude, albeit with some timing delay. The same analysis in the SD (Fig. 1D) rats showed that the b-wave was larger than expected in the older rats after allowing for a-wave amplitude changes.

**Photoreceptor Responses**

Figure 2A summarizes outer retinal findings by showing that the SD rats had a smaller saturated photoreceptor response than did the LE animals (F₁,₄₇ = 56.79; P < 0.0001) and that ageing significantly reduced Rm₂₃(F₁,₄₇ = 32.51; P < 0.0001). This ageing effect appeared to be greater in the SD rats (young, −644 ± 19 μV; old, −416 ± 20 μV; 35% decrease) than in the LE rats (young, −813 ± 36 μV; old, −699 ± 9 μV; 14% decrease). However, this difference between strains is not statistically significant (interaction term, F₁,₁ = 3.60; P = 0.06).

Photoreceptor sensitivity (S, Fig. 2B) was decreased by 0.31 log units in 18-month-old LE rats compared with their younger counterparts (young, 2.83 ± 0.03 log m²⋅cd⁻¹⋅s⁻³; old, 2.52 ± 0.04 log m²⋅cd⁻¹⋅s⁻³). Paradoxically, the older SD rats showed an improved photoreceptor sensitivity (0.18 log unit) compared with their younger counterparts (young, 2.88 ± 0.03 log m²⋅cd⁻¹⋅s⁻³; old, 3.06 ± 0.03 log m²⋅cd⁻¹⋅s⁻³). A two-way ANOVA revealed a significant interaction between age and strain (F₁,₁ = 45.04; P < 0.0001). The Bonferroni post hoc test confirms a significant decrease in the LE rats but a significant increase in the SD animals.

**Figure 1.** Group average ERG responses of 3-month (thin trace) and 18-month (thick trace) animals. (A) ERG at moderate to bright (−3.99 to 2.07 log cd⋅s⋅m⁻²) luminous energies for pigmented (LE) strain. (B) Normalized young and old responses in (A) to the saturated a-wave amplitude; inset: the a-wave leading edge at 2.07 log cd⋅s⋅m⁻² on an expanded time scale (E) shows the STR response of pigmented animals returned from dim luminous energies (−5.94 to −4.78 log cd⋅s⋅m⁻²) for LE. (F) The cone response at 2.07 log cd⋅s⋅m⁻². (C, D, G, H) Responses in the albino (SD) strain; other details as in (A, B, E, F).

**Figure 2.** (A) Average (± SEM) rod saturated amplitude (Rm₂₃) for young (◻) and old (◼) animals in the LE and SD groups, as labeled. *Statistical significance (P < 0.05) between the two age groups. (B) Rod sensitivity (S) on a log scale. Other details as in (A).
FIGURE 3. (A) Average (± SEM) energy–response function for P2 peak in young (○) and old (●) LE eyes. The symbols plot rod response for old LE rats at 2.07 log cd · s · m⁻², obtained in the twin-flash paradigm. *Dotted line: the hyperbolic function for the rod component of the data; *solid line: modeling of the cone component. (B) Average (± SEM) energy–response function for the P2 peak in young (○) and old (●) LE and SD eyes. (C) Average rod ON-bipolar maximum response (± SEM) in young (○) and old (●) LE and SD rats. (D) Average cone ON-bipolar maximum response (± SEM) in young (○) and old (●) LE and SD rats. (E) Average rod ON-bipolar k (± SEM) in young (○) and old (●) LE and SD rats. (F) Average cone ON-bipolar k (± SEM) in young (○) and old (●) LE and SD rats. *Statistical significance (P < 0.05) between the two age groups.

ON-Bipolar Cell Responses

Figures 3A and 3B show the ON-bipolar cell P2 amplitude plotted as a function of luminous energy for the young (open symbols) and old (filled symbols) LE and SD rats, respectively. The data were modeled by using two hyperbolic functions (Equation 2) to describe rod and cone bipolar cell responses. Figure 3C shows that the rod ON-bipolar cell P2 was smaller in the SD rats than in the LE rats (F₁,₄₇ = 63.3; P < 0.0001) and that the difference continued in older age (F₁,₄₇ = 6.5; P < 0.05) in both the LE (young, 1935 ± 79 μV; old, 1781 ± 79 μV; 8% decrease) and the SD rats (young, 1384 ± 56 μV; old, 1164 ± 47 μV, 16% decrease). There was no age and strain interaction (F₁,₁₁ = 0.2; P = 0.65), indicating a similar ageing effect in both strains.

Figure 3D shows that similar to the rod P2, the cone-mediated P2 was smaller in the SD rats than in the LE animals (F₁,₄₇ = 45.3, P < 0.001). Moreover, the cone P2 was reduced with age (F₁,₄₇ = 18.5, P < 0.0001) in both the LE (young, 718 ± 48 μV; old, 629 ± 13 μV; 13% decrease) and the SD rats (young, 546 ± 16 μV; old, 343 ± 17 μV, 37% decrease). There was no age and strain interaction (F₁,₁₁ = 2.8; P = 0.10).

As shown in Figure 3E, the rod bipolar cell semisaturation constant (k) had a significant strain and age interaction (F₁,₁₁ = 15.1; P < 0.001). The Bonferroni post hoc test revealed that rod P2 was more sensitive in the older SD rats than in the younger ones (young, −3.24 ± 0.02 log cd · s · m⁻²; old, −3.45 ± 0.03 log cd · s · m⁻²), but there was no age effect in the LE rats (young, −3.36 ± 0.03 log cd · s · m⁻²; old, −3.32 ± 0.02 log cd · s · m⁻²). Figure 3F shows a significant strain and age interaction (F₁,₁₁ = 24.5; P < 0.001) for cone P2 semisaturation. Again, post hoc testing revealed that the cone P2 semisaturation constant was reduced by age in the SD rats (young, −0.64 ± 0.03 log cd · s · m⁻²; old, −0.91 ± 0.04 log cd · s · m⁻²), but not in the LE rats (young, −0.85 ± 0.03 log cd · s · m⁻²; old, −0.73 ± 0.04 log cd · s · m⁻²).

Inner Retinal Responses

Figure 4A shows that the pSTR response was smaller in the SD rats than in the LE rats (F₁,₄₇ = 40.5; P < 0.0001). However, there was no difference between the young and old animals of either strain (LE young, 15 ± 1 μV; old, 13 ± 1 μV; 17% decrease. SD young, 6 ± 1 μV; old, 7 ± 1 μV; 19% increase), as confirmed by two-way ANOVA (interaction, F₁,₁₁ = 3.19, P = 0.08; ageing effect, F₁,₄₇ = 0.39, P = 0.54).

Figure 4B shows that the nSTR (Fig. 4B) was larger in the SD than in the LE rats (F₁,₄₇ = 9.01; P < 0.01). In addition, there was a significant reduction in the nSTR with age (F₁,₄₇ = 10.01; P < 0.01) that appeared to be greater in the older LE rats (young, −18 ± 1 μV; old, −11 ± 1 μV; 40% decrease) than in the SD rats (young, −21 ± 1 μV; old, −18 ± 1 μV; 12% decrease). However, there was no interaction between strain and age, as revealed by two-way ANOVA (F₁,₁₁ = 2.38; P = 0.13).

Identifying the Locus of Loss

Figure 5A shows the relative ratio of Vmax/Rm₁₄, pSTR/Vmax, and nSTR/Vmax for the young (open) and old (filled) LE rats. The ratio of Vmax/Rm₁₄ in the young eyes was 2.40 ± 0.07, which is not significantly different (P = 0.33) from that of the old LE rats (2.55 ± 0.08). This result means that, once the photoreceptor inputs were normalized in both ages, bipolar cell outputs were similar in the young and old LE rats, confirming the trend seen in Figure 1B. Similarly, the pSTR/Vmax ratio

FIGURE 4. (A) Average (± SEM) pSTR amplitude in young (○) and old (●) pigmented (LE) and albino (SD) rats. (B) Average (± SEM) nSTR amplitude in young (○) and old (●) LE and SD rats. *Statistical significance (P < 0.05) between the two age groups.
in the young LE rats (0.0077 ± 0.0004) is not significantly different (P = 0.53) from that of the old LE rats (0.0070 ± 0.0005). Therefore, when equated for rod bipolar cell input, the output of the ganglion cell pSTR was similar between the young and old animals. However, the nSTR/Vmax ratio in the young LE rats (0.0094 ± 0.0005) is significantly (P < 0.01) greater than that of the older cohort (0.0063 ± 0.0006), indicating a preferential nSTR attenuation with age, even after normalizing for rod bipolar cell input.

The same analysis was performed for the SD rats (Fig. 5B). The Vmax/Rmp3 ratio was significantly greater in the older cohort (young, 2.15 ± 0.051; old, 2.83 ± 0.081; P < 0.0001), which indicates that the bipolar cell output in the older SD rats was greater than expected, given the photoreceptor input (also see Fig. 1D). Similarly, ratio analysis showed that the pSTR output in older SD rats was better than expected, given the rod bipolar cell input (young, 0.0044 ± 0.0004; old, 0.0063 ± 0.0006; P < 0.05). However, the nSTR/Vmax ratio is not significantly different between the two age groups (young, 0.015 ± 0.00081; old, 0.016 ± 0.013; P = 0.81).

**Photoreceptor Versus ON-Bipolar Cell Sensitivity**

Figure 6 shows the average (±SEM) rod ON-bipolar cell sensitivity (1/k) against photoreceptor sensitivity (S) for both strains. In the pigmented animals, ageing produced a marked loss of photoreceptor sensitivity compared with little change in rod ON-bipolar cell sensitivity (circles). In contrast, the albino strain (squares) showed an age-related increase in photoreceptor sensitivity that was paralleled by a corresponding increase in rod ON-bipolar cell sensitivity.

**DISCUSSION**

At 3 months of age, the pigmented rats showed larger P3 and P2 amplitudes than did their albino counterparts, which is in concordance with previous studies. Despite smaller amplitudes, sensitivity was greater in the young albino rats than in the pigmented rats of the same age, which again agrees with previous work. This study shows that ageing resulted in markedly different outcomes in the pigmented and albino strains.

**The Effect of Ageing on Photoreceptor Responses**

Table 1 summarizes the age-related changes in ERG parameters in terms of the various cell classes they represent. In the pigmented rats, an ageing change from 3 to 18 months resulted in a 14% reduction in Rmp3 and a 0.31-log-unit phototransduction sensitivity decrease. In contrast, the older albino rats showed a photoreceptor amplitude decrease of 35%, but a sensitivity increase of 0.18 log units.

The reduced photoreceptor amplitudes found in older albino rats corresponds with results of Aleman et al., who reported an approximate 40% decrease in P3 amplitude in 18-month-old SD rats compared with that in 3-month-old animals. Similarly, Heiduschka and Schraermeyer compared ERG responses in Wistar (albino) and LE rats between 1.5 and 10 months and found that photoreceptor a-wave amplitudes of older animals decreased by 55% in the LE and 40% in the Wistar rats. Similar reductions in a-wave amplitude have been reported in older pigmented mice.41,45
This age-related decline in photoreceptor amplitude is likely to reflect a reduction in photoreceptors as well as shortening of the photoreceptor outer segments. Age-related photoreceptor loss may be greater in albino rats, as they are known to be more susceptible to light-induced damage. Although the lighting was maintained below 50 lux in our animal house and thus was unlikely to produce damage, this possibility cannot be ruled out entirely.

The Effect of Ageing on Rod and Cone ON-Bipolar Cell Responses

In the pigmented rats, there was a decline in both rod and cone ON-bipolar cell responses with age. These observations are consistent with the anatomic findings of Weisse, who reported a decrease in the number of bipolar cells with age. Furthermore, decreased photoreceptor input will also compound the decreased downstream ON-bipolar cell output. There was no significant difference in rod (−8% ± 4%) and cone (−13% ± 2%) ON-bipolar cell output with age (t = 1.00; P = 0.33).

In the older albino rats, cone ON-bipolar cell amplitude was 37% ± 3% smaller than their younger counterparts (t = 8.72; P < 0.001). The relative decrease in amplitude is significantly greater (t = 4.54; P < 0.001) in cone than in rod ON-bipolar cell responses (16% ± 3%), implying a greater age-related deficit in cone responses in albino rats. Consistent with our findings, Chrysostomou et al. reported that cone b-wave amplitudes are more reduced (~40%) than the change in rod b-wave amplitudes (~50%) between with age in SD rats. The greater reduction in the cone b-wave compared to the rod b-wave might arise due to less redundancy in the rat cone pathway. Szel and Röhlich reported that cones make up only a small proportion (0.85%) of rat photoreceptors. Given the relative paucity of cones, fewer cones may be lost before a significant reduction in cone bipolar cell responses is detectable. Thus, for the same relative reduction in rod and cone photoreceptors, a greater reduction in cone bipolar cell output (i.e., the cone P2) may be expected. Why this preferential decrease in cone b-wave is not seen in pigmented rats is not clear, but one possibility is that cone photoreceptors in pigmented rats may be more resistant to light-induced damage than are albino cone photoreceptors.

In terms of rod and cone ON-bipolar cell sensitivities, Figures 3E and 3F show that ageing affected the two rat strains differently. In albino rats, there was an improvement in cone ON-bipolar cell sensitivity of 0.26 ± 0.04 log units in the old compared with the young eyes, which is not significantly different from the increase seen for rod ON-bipolar cell sensitivity (0.21 ± 0.03; t = 1.15, P = 0.26). Given this similar magnitude of change, it is likely that the improved sensitivities of both rod and cone ON-bipolar cell function reflect a downstream effect of phototransduction sensitivity improvement seen in the older albino rats. On the other hand, in pigmented rats, despite a reduced photoreceptor sensitivity there appears to be a preservation of both rod and cone ON-bipolar cell sensitivity (log unit change relative to young animal rods 0.04 ± 0.02 and cones 0.12 ± 0.04; t = 1.47; P = 0.16).

The Effect of Ageing on Inner Retinal Responses

Figures 4A and 4B show that in the pigmented rats, the nSTR appeared to be much more decreased with age than did the pSTR. Studies in rats have shown that the pSTR largely reflects the response of retinal ganglion cells, whereas the nSTR receives contributions from both retinal ganglion cell and amacrine cell responses. Given that anatomic studies have demonstrated that the number of both ganglion and amacrine cells decrease with age, it is likely that the combined

| Table 1. Summary of Age-Related Changes in Amplitude and Sensitivity of ERG Parameters |
|--------------------------------|---------------------------------|---------------------------------|
| **Photoreceptors** | **Long-Evans (Pigmented)** 18 vs. 3 months (%) | **Sprague-Dawley (Albino)** 18 vs. 3 months (%) |
| Maximum response | 14 ↓ 0.31 log units ↓ 0.31 log units ↓ 0.18 log units ↑ |
| Rod bipolar cells | 8 ↓ 0.04 log units ↓ 0.21 log units ↑ |
| Cones bipolar cells | 13 ↓ 0.12 log units ↓ 0.026 log units ↑ |
| Retinal ganglion cells | pSTR response 17 ↓ 19 ↑ |
| nSTR response | 40 ↓ 12 ↓ |

Figures 6. Average (±SEM) of rod ON-bipolar cell sensitivity against photoreceptor sensitivity in young (○) and old (●) pigmented animals. Bold solid line: rate of change between the two ages; average (± SEM) in young (●) and old (○) albinos. Dashed line: rate of change between the two ages. Thin solid line: a 1:1 relationship between S and 1/k (a rod S change is manifest as a similar change in ON-bipolar cell 1/k).
loss of the two cell classes will result in a greater decrease in nSTR. In old albino rats, both pSTR (+0.21%; P = 0.27) and nSTR (−12%; P = 0.18) were not significantly different than in the younger cohort. This preservation of inner retinal function is likely to arise from the observed improvement in phototransduction sensitivity and in turn improved ON-bipolar cell sensitivity, despite the fact that age-related reductions in the number of amacrine and retinal ganglion cells are likely to be present.23,24,53 Our finding of a relative preservation of ganglion cell function in senescence suggests the presence of age-related adaptations57–59 to overcome structural and anatomic decline.60

Age-Related Retinal Plasticity and Sensitivity Changes

Figure 6 shows that, when compared to their young counterparts, the old albino rats showed an increase in sensitivity of similar magnitude in both photoreceptor and ON-bipolar cell responses. The similarities in magnitude suggest that the up-regulation of ON-bipolar cell sensitivity can be explained as a downstream effect of increased photoreceptor sensitivity in albino rats.

The increase in photoreceptor sensitivity in the albino rats may be attributable to alterations in photoreceptor structure. Westbrook et al.61 reported an increased cone sensitivity in form-deprived myopic chickens. They suggest that the longer axial length of myopic eyes produces an increase in cone outer segment diameter, thus increasing the photon-collecting area, resulting in greater sensitivity. It may be that the improved sensitivity observed with age in the albino rats arises from an increase in photoreceptor diameter to fill the spaces left by photoreceptors lost with age. This concept has been suggested by Curcio et al.62 and would account for the resultant increase in phototransduction sensitivity despite the decrease in amplitude (most likely owing to photoreceptor loss) in albino rats. If this were the case, then why would such an age-related increase in phototransduction sensitivity not be seen in older pigmented rats? It may be that at 18 months of age, there is less photoreceptor loss in the pigmented rats, probably because of a higher capacity to resist light-induced damage, as evidenced by a smaller decline in photoreceptor amplitude (Fig. 2A). Consistent with this idea, Li et al.63 reported that there is little change in photoreceptor density in older pigmented mice. Thus, in pigmented eyes there may be no change in the outer segment collecting area. Indeed, the pigmented rats showed a decrease in phototransduction sensitivity, consistent with single-cell recordings from aged pigmented mice by Kolesnikov et al.,45 who suggested that the reduction in sensitivity arises from an age-related increase in the dark noise of photoreceptors.

It is worth noting that the reduction in phototransduction sensitivity in the older pigmented animals can also arise from reduced retinal illuminance, due to a smaller pupil size on dilation and/or age-related media opacities. Measurement of pupil size in pigmented rats 20 minutes after instillation of mydratics showed that pupil diameter was actually larger in the old eyes than in the young eyes (n = 6 each group; old, 4.5 ± 0.2 mm, versus young, 3.7 ± 0.2 mm; P < 0.05). Thus, it appears unlikely that an age-related reduction in the efficacy of mydriatic agents in producing dilation underlies the observed decline in phototransduction sensitivity.

Another possible cause of the age-related decline in sensitivity is that older rats have increased lens opacities. Alpern et al.64 showed in a combination of albino and pigmented rats that increased lenticular light absorption occurs in 6-month-old rats compared with 15-day-old animals. Other studies have reported lens changes at 18 months of age in albino species, including Wistar65–66 and Fischer67 rats. However, pigmented rats are less likely to have developed significant lens changes by 18 months of age.69 Nevertheless, age-related increased media density can produce a neutral density effect, which reduces retinal illuminance. To address this possibility, we consider the rate of saturation of the a-wave, that is, when Equation 1 is modeled to individual waveforms phototransduction sensitivity decreases at high light levels (see Fig. 6 in Lamb and Pugh12 and Fig. 5 in Breton et al.70). With this analysis (see the Supplementary Material and Supplementary Fig. S1 for more detail, http://www.iovs.orglookup/suppl/doi:10.1167/iovs.11-7602/-/DCSupplemental), a reduction in sensitivity due to a cataract-related reduction in retinal illumination would be overcome by increasing the luminous energy, yielding a common maximum rate of increase in amplitudes of ON-bipolar cell responses. Equation 1 to individual waveforms showed that the maximum rate of increase was lower in the old pigmented rats, which argues against reduced retinal illuminance in the 18-month LE rats. This finding is consistent with the analysis by Cicciayan and Jacobson,71 showing that the age-related decline in phototransduction sensitivity is greater than would be predicted by lens changes.

Despite the reduction in phototransduction sensitivity, ON-bipolar cell sensitivity is normal in older LE rats, a finding that suggests that some adaptation has occurred to preserve ON-bipolar cell sensitivity in the older pigmented rats. This improvement in ON-bipolar sensitivity produces a normal ON-bipolar cell output despite the substantially decreased photoreceptor amplitude (Figs. 2A, 2B). That ON-bipolar cell sensitivity can change independent of phototransduction sensitivity has recently been demonstrated in human infants with retinopathy of prematurity.72 Liets et al.73 provided anatomic evidence of ON-bipolar cell plasticity by showing that rod bipolar cell dendrites can grow into the outer nuclear layer to make contact with rod photoreceptor spherules in normal aged mice. In addition, Terzibasi et al.65 showed that rod bipolar cell and horizontal cell dendrites increased steadily between 12 and 24 months of age in mice (C57BL/6J strain). Finally, Aleman et al.43 proposed that changes at rod–bipolar cell synapses can compensate for the reduction in the number of photoreceptors in senescence. These studies are consistent with our findings that the pigmented retina has a capacity, at the bipolar cell level, to maintain functional output with aging.

**Summary**

This study provides functional evidence of retinal plasticity in response to age-related dysfunction in both albino and pigmented rats, albeit of different retinal cells. In albino animals, the age-related adaptation appears to occur at the photoreceptors, producing an increased sensitivity that then manifests as a relative sparing of bipolar and ganglion cell signals. In pigmented animals, the age-related adaptation seems to occur at the photoreceptor to ON-bipolar cell level, producing a normal bipolar cell and ganglion cell response (pSTR) in the presence of lower photoreceptor output. As albino and pigmented rats exhibit different ageing phenotypes, careful consideration of the rodent strain is needed in ageing studies. Human ageing data74,75 show decreased amplitudes with delayed implicit times in both photoreceptor and ON-bipolar cell responses, which correlates better with our findings in the pigmented LE strain. Hence, pigmented rats appear to be a better model for studying age-related changes in the ERG of human eyes.

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References


