·Basic Research·

Comparison of electroretinographic responses between two different age groups of adult Dark Agouti rats

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Received: 2014-12-23 Accepted: 2015-02-05

Abstract

• AIM: To describe and compare the differences in electroretinographic responses between two different age groups of adult Dark Agouti (DA) rats and to better understand the effect of age on retinal histology and function.

• METHODS: The electroretinographic responses of two different age groups of adult DA rats were compared. Animals were divided into younger adult DA rats 10–12wk (n=8) and older adult DA rats 17–19wk (n=8). Full field electroretinography (ERG) was recorded simultaneously from both eyes after dark adaption and light adaption and parameters including the positive scotopic threshold response (pSTR), negative scotopic threshold response (nSTR), scotopic a–wave, b–wave, photopic a–wave, b–wave and photopic negative response (PhNR) were compared between groups.

RESULTS: The older adult rats displayed lower stimulation thresholds of the STRs (pSTR and nSTR) and higher amplitudes of pSTR, scotopic a–wave and b–wave, photopic b–wave and PhNR amplitudes, with shorter implicit times. Photopic a–wave amplitudes were however higher in the younger adult rats.

• CONCLUSION: In summary, for the rod system, photoreceptor, bipolar cell and RGC activity was enhanced in the older adult rats. For the cone system, RGC and bipolar cell activity was enhanced, while photoreceptor activity was depressed in the older adult rats. Such age–related selective modification of retinal cell function needs to be considered when conducting ophthalmic research in adult rats.

• KEYWORDS: electroretinographic responses; Dark Agouti rats; age

DOI:10.3980/j.issn.2222–3959.2015.05.08

Fu L, Lo AC, Lai JS, Shih KC. Comparison of electroretinographic responses between two different age groups of adult Dark Agouti rats. *Int J Ophthalmol* 2015;8(5):898–903

INTRODUCTION

Experimental models for ophthalmic disease in rats are important in the study of mechanisms of disease as well in the development of novel treatment strategies. Previous research work has shown that maturation and aging causes a significant change in different retinal cell count in both humans and rodents. Over time, there was a demonstrated reduction of rod photoreceptors and retinal ganglion cells (RGC)[1-3]. The retinal nerve fiber layer thickness (RNFLT) also decreased over time and reaches maximum thinning after 50 years old in humans [4,5]. However, there is an apparent dearth of literature when comparing responses between groups with smaller age differences.

The electroretinogram (ERG) is a test of retinal function and is widely used in clinical practice and research. In 1986 Holmgren [6] first noted an oscillation of electrical potential when light stimulated an amphibian eye. In clinical practice, a-waves and b-waves are the most frequently used components, in which the a-wave reflects the function of the photoreceptors and the b-wave reflects the integrity of bipolar and Müller cells[7,8]. In addition to this, the scotopic threshold response (STR) and photopic negative response (PhNR) are important components in the assessment of RGC function in animal studies [9-14]. Although less utilized in clinical electroretinography, these parameters are increasingly important in animal glaucoma models[15,16]. The amplitude and implicit time of ERG components can be affected by various factors, including strain, age and gender. Thus age-specific electroretinographic norms should be established for rodent strains used in models of ophthalmic disease.

The Dark Agouti (DA) rats have been widely used in visual science research, serving as models for diabetic retinopathy [17,18], glaucoma [19] and novel ophthalmic treatments[20,21]. The eyes of DA rats are pigmented, thus they serve as a suitable model for human eyes. Furthermore, DA rat eyes protrude from their orbital rims, allowing ease of access during experimental ophthalmic procedures.

Here the authors investigate the electroretinographic responses in two different age groups of adult DA rats to offer an age-specific reference on retinal function.

MATERIALS AND METHODS

Materials All animals were kept in a temperature-controlled room and subjected to a 12-hour light/12-hour dark cycle. They were provided with a water supply and sufficient food. Animal care and experimental methods conformed to the Association of Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research and were approved by the Committee on the Use of Live Animals in Teaching and Research (CULATR) of the University of Hong Kong. The animals were divided into two groups: younger adult DA rats 10-12wk old (n=8) and older adult DA rats 17-19wk old (n=8). Both age groups have been commonly used in ophthalmic research.

Methods

Electroretinography Animals were dark adapted for more than 12h and anesthetized with a mixture of intraperitoneal ketamine and xylazine (100 mg/kg and 10 mg/kg; Alfasan International, Woerden, the Netherlands) before the procedures. Experiments were carried out under dim red light in the dark room. The eyes were further anaesthetized with 1-2 drops of proxymetacaine hydrochloride (0.5% Alcaine; Alcon, Fort Worth, TX, USA) and the pupils were dilated with 1-2 drops of tropicamide (1% Mydriacyl; Alcon). ERG signals were recorded *via* a noninvasive contact lens electrode with a gold ring mounted in the inner surface (Mayo, Japan) using an Espion Diagnosys system (Diagnosys LLC, Littleton, MA, USA). A needle electrode was inserted into the subcutaneous tissue between the ears to serve as a negative reference and another needle electrode was inserted into the subcutaneous tissue at the lower back to serve as a ground reference. Both eyes were stimulated simultaneously. Body temperature was maintained at 37℃ with a heating pad throughout the procedure (Figure 1).

Recording protocol The recording protocol was modified from those previously reported by Bui *et al* [22] on Brown Norway rats and Chrysostomou and Crowston [14] on the C57BL/6J mice. The DA rats were dark adapted for 10min again before stimulation. For the STR responses, LED peak flashes of 4ms were delivered through the ColorDome Ganzfeld System (Diagnosys, Lowell, MA, USA). The intensities ranged from -4.7 to -2.37 log cd.s/m^2 in 0.2 log unit increments. Twenty-five responses with an inter-stimulus interval (ISI) of 2s were obtained per intensity for averaging of results. For the scotopic a wave and b-waves, single flashes from xenon arc light with intensities ranging from -1.5 to 2.7 log cd.s/m^2 in 0.3 log unit increments were used and the ISI was progressively lengthened from 10s to 60s. After 15min of adaptation to a 40.0 cd.s/m^2 rod saturating green background, 25 responses per intensity within ISI of 2s were triggered by a xenon arc light from 1.22 to 2.72 log cd.s/m^2 in 0.5 log unit increments.

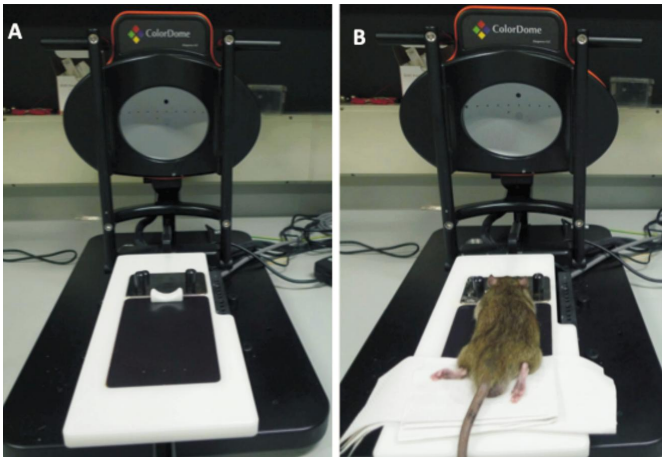

Figure 1 Experimental setup A: ColorDome Ganzfeld System (Diagnosys, Lowell, MA, USA); B: The animals were placed black heating pad and connected with electrodes.

Waveform analysis For the STR, a-wave, b-wave and PhNR recordings, both amplitudes and implicit times (IT) were measured after defining the maximum peak and trough with respect to the baseline. For each stimulation intensity level tested, only responses with a peak or trough were analyzed. For measurements of STR, only waveforms with a positive peak followed by a negative trough were analysed in this study and signals were recorded with band-pass filters from 0.3 to 30 Hz and digitized at 1 kHz. For other responses, band-pass filters from 0.3 to 500 Hz were used and signals were digitized at 2 kHz.

Statistical Analysis Results were presented as mean ± standard error of measurement (SEM). The differences between younger adult and older adult DA rats were compared using Student's t-test (Prism 5; GraphPad Software Inc., San Diego, CA, USA). A P value of less than 0.05 was considered to be statistically significant.

RESULTS

Scotopic Threshold Responses The stimulation thresholds of pSTR and nSTR waves in the older adults were both 0.2 log cd.s/m^2 lower than in the younger ones, while the amplitudes of pSTR waves were higher in the older rats at all tested intensities. The amplitudes of nSTR waves were only higher in the older rats when stimulated at the intensity of -3.9 log cd.s/m^2 and -3.1 log cd.s/m^2 (Figure 2). At each stimulation intensity level tested, there were a higher number of pSTR waves recorded than nSTR waves (Figure 3). It was observed that a higher number of pSTR waves was recorded as stimulation intensity increased and reached a maximum response number of 16 in both age groups at intensities ≥-3.3 log cd.s/m^2. A similar trend was not observed for nSTR waves.

There were no differences in IT between the two age groups under lower stimulation intensities. However IT of the older adults were significantly shorter than the young ones under the higher intensities of -3.1, -2.9, -2.7 log cd.s/m^2 with

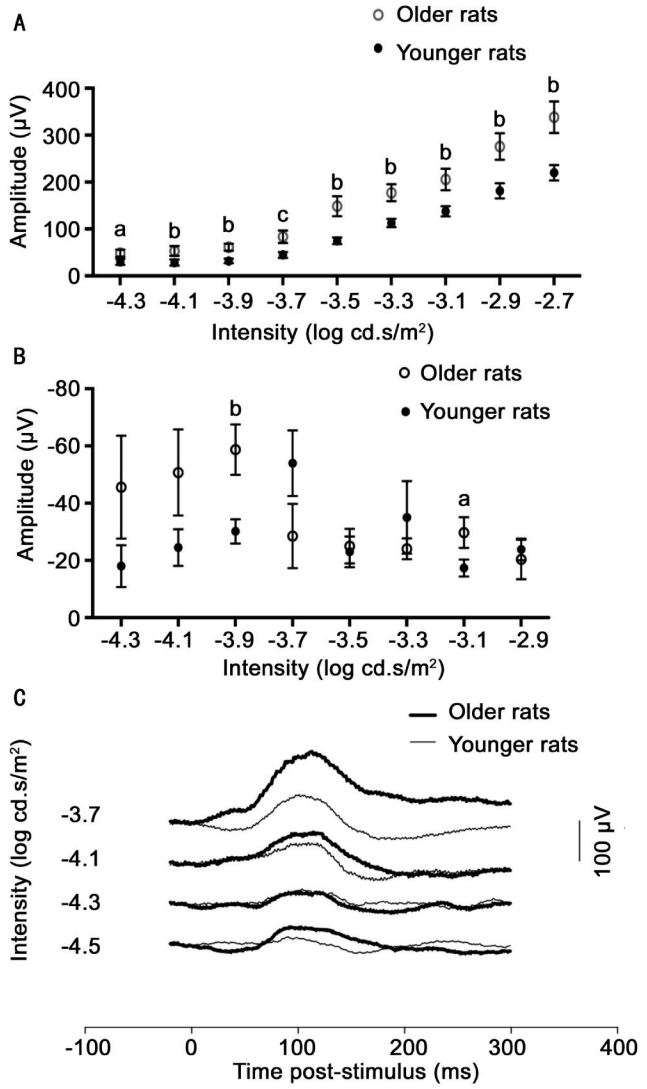

Figure 2 Comparison of amplitude of the STRs in the younger and older adult rats A: Higher response amplitudes of pSTR here recorded in the older rats compared to younger ones; B: The amplitudes of the nSTR in older rats were only significantly higher than younger ones under intensities of -3.9 and -3.1 log cd.s/m^2; C: Individual examples showed the STR waveforms under four different intensities. $^aP<0.05$; $^bP<0.01$; $^cP<0.001$.

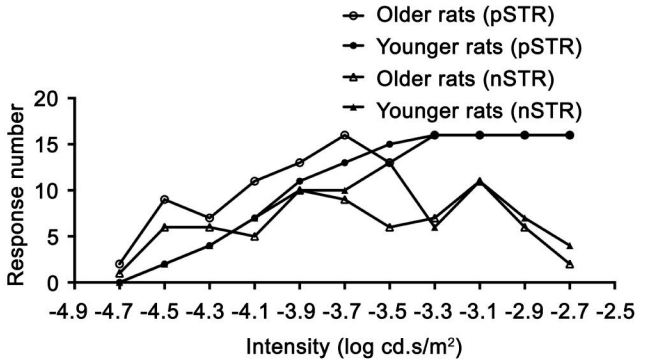

Figure 3 Response number of the STRs at all intensities More frequent pSTR recordings were seen in the older rats compared to younger ones and more pSTR recordings were made compared to nSTR recordings at each intensity. The nSTR number at increasing intensities showed no definite trend. On the other hand more frequent pSTR recordings were seen as the intensity increased and reached 100% response at intensities higher than -3.5 log cd.s/m^2.

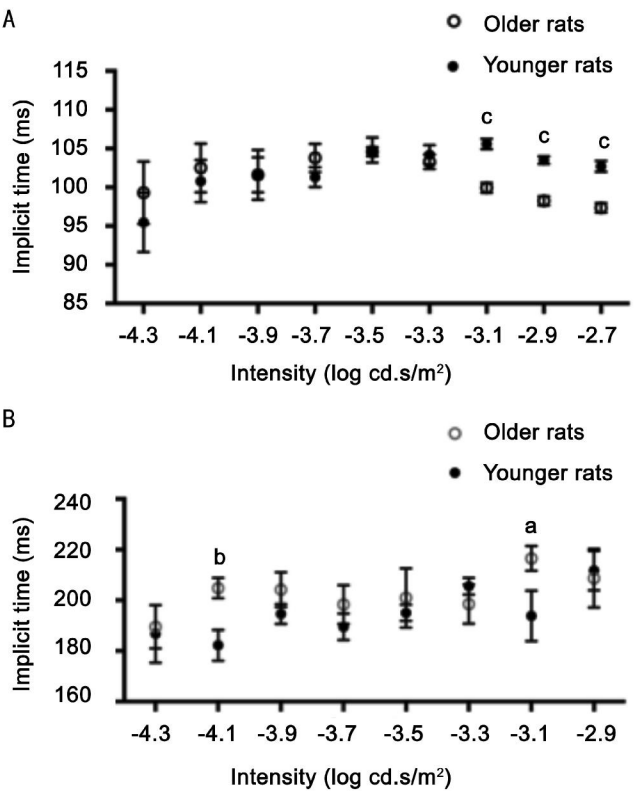

Figure 4 Comparison of IT of the STRs in the younger and older rats A: At the higher three intensities of stimulation, older rats had faster pSTRs than the younger ones. In the lower intensities, there were no significant differences between the ITs of the two age groups; B: The nSTR of older rats had longer ITs both under a lower intensity (-4.1 log cd.s/m^2) and a higher intensity stimulation (-3.1 log cd.s/m^2). $^aP<0.05$; $^bP<0.01$; $^cP<0.001$.

differences of -5.70±0.9170ms, -5.355±0.9463ms and -5.244±0.7745ms respectively (Figure 4). This finding may suggest that older adults are more sensitive to higher intensity stimulations. However, IT of the nSTR waves were longer in the older adults for all stimulation intensities. They were 22.64±7.308ms and 22.75±11.11ms longer than the ITs in young adults when stimulated by the intensities of -4.1 and -3.1 log cd.s/m^2. The implicit time differences in the nSTR waves were larger than those of pSTR waves.

Scotopic a-waves and b-waves In both age groups, the amplitude of a-wave responses rose significantly with increasing stimulation intensity. The largest measured response was ten folds that of the smallest one. The a-wave amplitudes were significant higher in older adults at intensities ranging from -0.3 to 2.4 log cd.s/m^2. In both age groups, the amplitude of b-waves also rose with increasing intensity, but with the maximal b-wave amplitude being only 2 folds that of the smallest response. The b-wave amplitudes were significantly higher in older adult rats across all tested stimulation intensities (Figure 5).

In both age groups, ITs of a- and b-waves shorted as the stimulation intensity increased. For stimulation intensities lower than 0 log cd.s/m^2, the older adult rats responded with

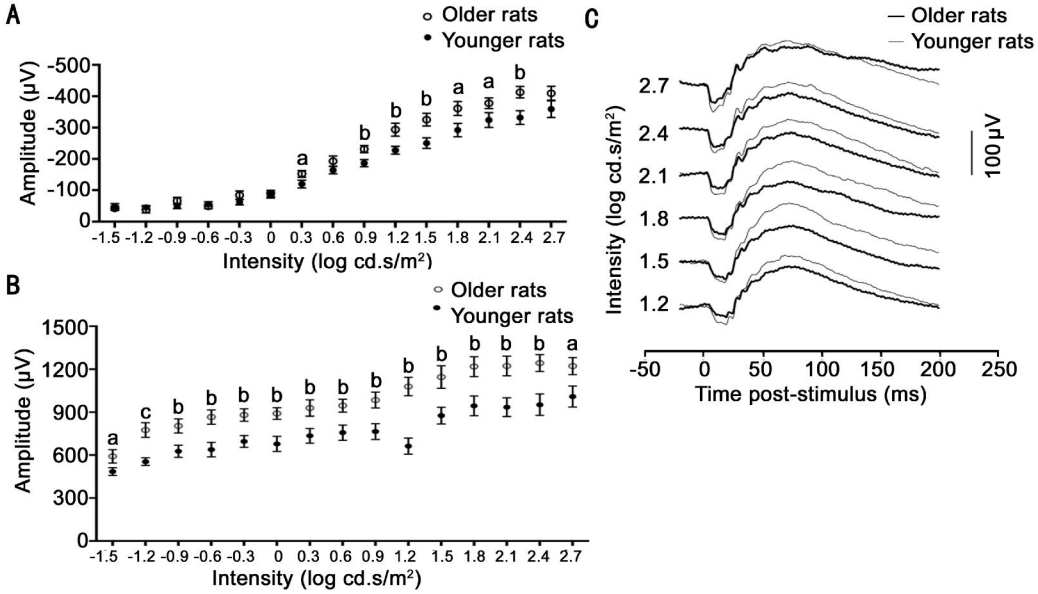

Figure 5 Comparison of amplitude of the scotopic a-wave (A) and b-wave (B) in the younger and older rats Higher amplitudes of scotopic a and b-waves were noted in the older adults. The amplitudes of scotopic a-wave and b-wave increased as stimulation intensity rose. C: Representative examples (displayed the waveforms stimulated by 6 intensities). [a] $P<0.05$; [b] $P<0.01$; [c] $P<0.001$.

significantly shorter ITs than the younger adult rats (Figure 6).

Photopic a-waves, b-waves and PhNR Waves For both age groups, there was a less dramatic rise in amplitude for photopic a- and b-wave responses with increasing stimulation intensity than that which was seen with the scotopic responses. Furthermore, unlike scotopic responses, the amplitudes of photopic a-waves were significantly lower in older adult rats than in younger ones. However, the amplitudes of photopic b-wave of the older rats were significantly higher than that of the younger ones (Figure 7). For the PhNR waves, the response amplitude rose with increasing intensity and reached a peak at 1.72 log cd.s/m^2, and decreased with higher amplitudes thereafter. The older adult rats were noted to have significantly higher PhNRs than younger ones (Figure 8).

The ITs of the a-wave responses progressively shortened with increasing intensities in both groups. However, the reverse was observed in both groups for the ITs of the b- and PhNR wave. Both ITs of b- and PhNR waves lengthened with the increasing intensities. Between groups, only the ITs of the a-wave of the older adult rats were significantly shorter than the younger adults at all tested stimulation intensities.

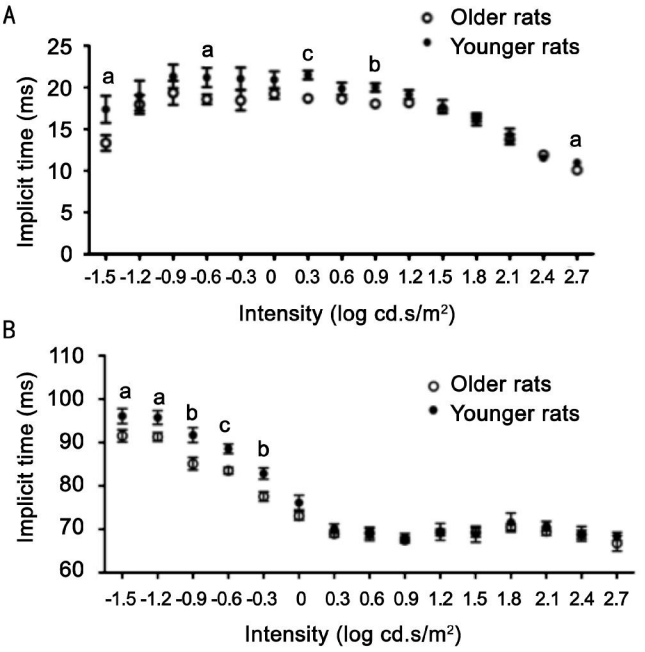

Figure 6 Comparison of IT of the scotopic a-wave (A) and b-wave (B) in the younger and older rats The older rats displayed shorter ITs of scotopic a-wave at certain intensities and shorter ITs of b-wave below stimulation intensity of 0 log cd.s/m^2. ITs of the scotopic a-wave and the b-wave shortened as the intensities increased. [a] $P<0.05$; [b] $P<0.01$; [c] $P<0.001$.

DISCUSSION

In our experiments, older adult rats were shown to have lower response thresholds for pSTR and nSTR waves as well as higher stimulation amplitudes for pSTR waves at all tested intensities. However the older group had higher nSTR amplitudes only at lower stimulation intensities (-3.9 log cd.s/m^2 and -3.1 log cd.s/m^2). It is thus likely that the retina of older adult rats is more sensitive to dim flashes than that of younger rats. Furthermore both scotopic a- and b-wave amplitudes were higher in the older adult rats. As discussed, the scotopic a-wave is the product of rod photoreceptor activity, while the scotopic b-wave reflects the rod-driver bipolar cell and Müller cell activity. It is thus possible that older adult rats have either a higher rod photoreceptor, rod-driven bipolar and Müller cell count or alternatively they may have superior rod system function.

However, after light adaptation, the trend of age-related enhancement was less clear. The amplitudes of photopic a-waves were lower in the older adults, while the amplitudes

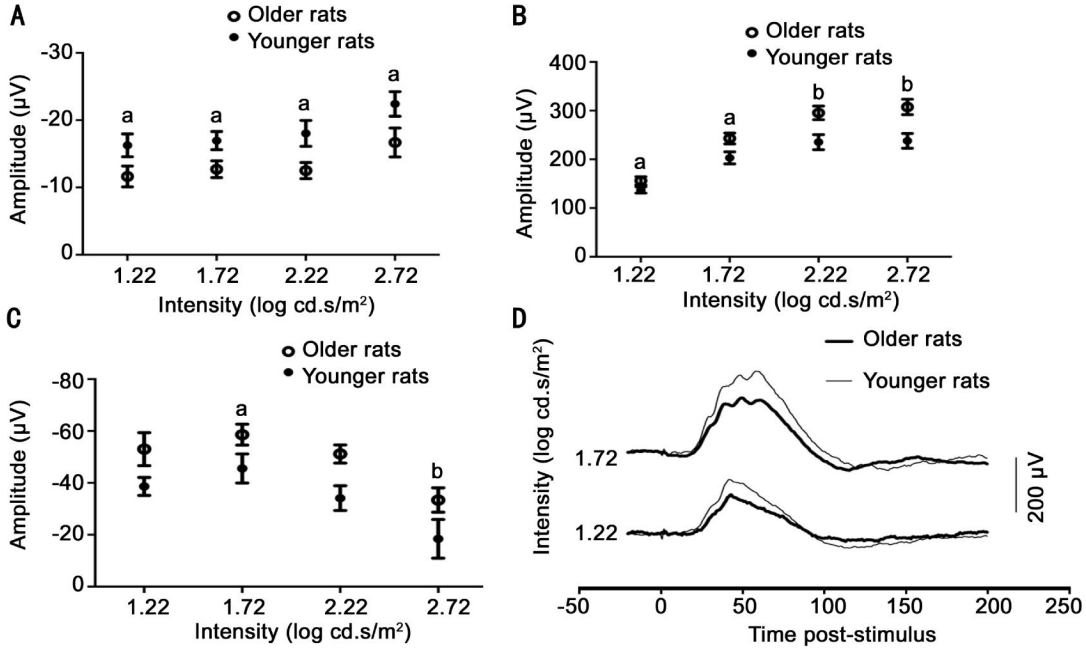

Figure 7 Comparison of amplitude of the photopic a-wave (A), b-wave (B) and PhNR (C) in the younger and older rats At all intensities, older rats had lower photopic a-wave amplitudes and higher photopic b- wave amplitues than the younger rats. For PhNRs, their amplitudes were higher in older under the lower three intensities and reached no significant difference at stimulation intensity of of 2.72 log cd.s/m^2. D: The representative examples was stimulated by lower two intensities. $^aP<0.05$; $^bP<0.01$.

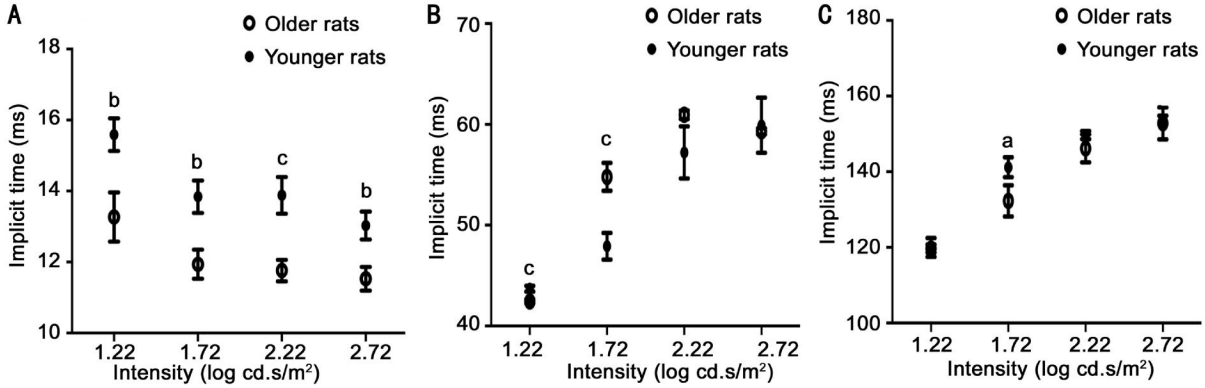

Figure 8 Comparison of implicit times of the photopic a-waves (A), b-waves (B) and PhNRs (C) in the younger and older rats For a-waves, the older rats responded sooner than the younger rats at all intensities and for b-waves, they were faster at a stimulation intensity of 1.22 log cd.s/m^2 but slower at a stimulation intensity of 1.72 log cd.s/m^2. There were nearly no differences between the two groups in the ITs of the PhNR except the response was faster in older rats at the intensity of 1.72 log cd.s/m^2. $^aP<0.05$; $^bP<0.01$; $^cP<0.001$.

of b-wave and PhNR waves were lower in the younger adults. The ITs of the photopic a-waves were shorter in the older adult but were variable in the photopic b-waves and there were no significant differences between age groups for PhNR waves. Thus, while the cone photoreceptor cells in the older rats may be more sensitive than that of younger adults, the response speed of other cells under photopic conditions were not significantly different between the two age groups. Thus age-related changes in cone-related histology and function is likely to be different to the trend observed in the rod system. It is also important to note that age-related changes in STR waves were not consistent with that of PhNR waves. Both waves have previously been cited to reflect inner retinal layer activity. Inconsistency in correlation between the two waves may suggest that they are unreliable markers for use in glaucoma models.

As illustrated from our results, a small age-difference of 7wk has already resulted in selective enhancement of all cells in the rod system and of the cone-driven bipolar cell and RGC function in the cone system. Conversely, cone photoreceptor activity is diminished in the older rats. Furthermore, to our knowledge, this is the first study show the effect of age on the STR and PhNR wave recordings. In our study, the age of the rats we used is equivalent to the predominant age groups of our glaucoma patients[23,24]. The prevailing conclusion from previous animal and human studies was that aging results in a reduction of response amplitudes on electroretinography [25]. Rod photoreceptor count has been shown to decrease by 30% over a lifespan. In studies on mice, cone photoreceptors exhibited a 20% age-related selective cell loss of medium/long wavelength cones in the peripheral ventral area over a lifetime [3,26]. This was suspected to be the cause of

visual deterioration with age in mouse models[27]. In addition, there is an observed reduction in retinal nerve fiber layer thickness with age in humans [4,5]. Such studies are however looking at age differences of more than 10 human years apart. Animals of such extremes of age are not usually used in experiments. Our study is the first to report changes between animals of smaller age differences. In our study, the age groups of 10-12wk and 17-19wk are both commonly used in ophthalmic research as a single homogenous group, making our results important and relevant to investigators conducting research on DA rats[28-30].

This is the first study to report age-related differences in key functional parameters involving including photoreceptor, bipolar cells, and the inner retinal layers of the retina in DA rats. The results demonstrate enhancement of the visual pathway through maturation.

The study illustrates the importance in considering age-related changes in retinal function when interpreting electroretinographic results from animal studies. A relative value with reference to age-matched norms will be much more useful than absolute values. Further studies should be performed to investigate the histological differences in the two age groups, including retinal morphology and differential cell count, in order to understand the underlying reasons for the different electroretinographic responses.

ACKNOWLEDGEMENTS

The paper was presented as a poster presentation at the 2nd Asia-Pacific Glaucoma Congress held between the 26th -28th of September 2014 in Hong Kong.

Foundation: Supported by the Seed Grant for Basic Research, Research Services, The University of Hong Kong.

Conflicts of Interest: Fu L, None; **Lo AC,** None; **Lai JS,** None; **Shih KC,** None.

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