Original Paper

Variability of Normal Values of Electroretinogram Parameters Due to Aging in Healthy Individuals

ANDREEA C. CORÎCI[1], D.O. ALEXANDRU[2], OANA M. CORÎCI[1], MIHAELA PUIANU[3], MARIA IANCĂU[1], A. ŞTEFĂNESCU-DIMA[3]

[1]*University of Medicine and Pharmacy of Craiova, Faculty of Medicine, Department of Physiology*
[2]*University of Medicine and Pharmacy of Craiova, Faculty of Medicine, Department of Medical Informatics and Biostatistics*
[3]*University of Medicine and Pharmacy of Craiova, Faculty of Medicine, Department of Ophthalmology*

ABSTRACT: Aim- To find normal values of implicit time and amplitudes of full-field electroretinogram and to determine their changes with age in healthy Romanian subjects. **Material and Methods**- The prospective study included 59 healthy subjects aged between 20 and 80 years old; in the end, we had valid ERG recordings for all tests from 54 subjects. All of the participants underwent full-field ERG, recorded with Metrovision MonPack One system. The implicit times and amplitudes were analyzed for a and b waves in dark-adapted 0.01 ERG, dark-adapted 3.0 ERG, dark-adapted oscillatory potentials, light-adapted 3.0 ERG, and 30Hz flicker ERG according to International Society for Clinical Electrophysiology of Vision (ISCEV) protocols. **Results-** ERG latency values were bigger in subjects above 50 years old than in younger subjects for b wave in dark adapted 0.01, dark adapted 3.0, light adapted 3.0 and dark adapted 3.0 flicker and for a wave in dark adapted 0.01 and dark adapted 3.0 ERG. There was no significant difference in latency values for dark adapted 3.0 oscillatory potentials between young and old subjects. Because of increased variability, we could not prove that observed differences for amplitudes held statistical significance. **Conclusions-** This study proves there is a major loss in retinal activity due to aging, most of it being caused by the rod cells delayed response. Also, oscillatory potentials do not seem to be affected by age, and could prove a valuable test to investigate for changes in patients with Diabetes mellitus.

KEYWORDS*: ERG, healthy subjects, Romania, implicit time*

Introduction

The global, Ganzfeld or full-field electroretinogram (ERG) is a test used to assess the status of the retina in eye diseases in humans, and also in laboratory animals used as models of retinal diseases [1]. The recording is made between an active electrode either in contact with the cornea or conjunctiva, or a skin electrode placed just below the lower eyelid margin and a reference electrode, placed near each orbital rim or on the forehead

Unfortunately, the use of ERG in Romania is limited, and there are only a few studies based in our country addressing this issue [2]. Therefore, we believe it is necessary to evaluate the ERG parameters for an ophthalmologically healthy sample group of Romanian subjects, and to create a valid reference, based on internationally-approved clinical protocols.

For the ERG to be an effective tool in assessing normal and pathological retinal activity, it is important that the contributions of the various retinal cells be well characterized [3]. The most important and studied components of ERG are the a and b waves, but there are many other elements, such as oscillatory potential (OP), c, d and m wave, scotopic threshold response (STR) and early receptor potential (ERP), each representing a certain retinal cell activity [4]. The a-wave shows the activity of the photoreceptors in the outer retina and the b-wave reflects the activity of ON bipolar cells and the Muller cells [1,4]. Alterations of these structures are translated through amplitude, latency and morphology changes of a and b waves.

Since 1989, the International Society for Clinical Electrophysiology of Vision (ISCEV) has continuously attempted to standardize the procedures in order to obtain universally comparable responses [5,6,7,8], the last update being issued earlier this year [9].

In this study, we aim to evaluate ERG normal values and variability due to ageing, in an ophthalmologically healthy Romanian population, based on ISCEV recommendations available at the beginning of the study [8]. To this end, we tried to find normal values for implicit time and amplitude for all standard tests of full-field ERG for subjects of ages between 20 and 80 years, and to analyze their changes with age.

The ISCEV Standard ERG includes the following responses, named according to the condition of adaptation and the stimulus: dark-adapted 0.01 ERG (response to dim stimulation in dark adaptation, which evaluates rods

response), dark-adapted 3.0 ERG(response to bright stimulus in dark adaptation, which analyses mixed rod-cone response), dark adapted 3.0 oscillatory potentials ERG, light adapted 3.0 ERG (response to a bright stimulus in light adaptation, which shows single flash cone response) and light adapted 3.0 flicker ERG (response to a flickering stimulus in light adaptation [8] Even if the ISCEV protocol is strictly followed, there are certain factors that may change the values of the ERG parameters, such as age, sex, pupil dilatation, media clarity, light intensity, state of adaptation. As such, each electrophysiology laboratory is advised to produce normal values for its own equipment and patient population [8].

Material and Methods

Subjects

We recorded ERG in 59 ophthalmologically healthy subjects, selected among patients' attendants or subjects who perform routine eye evaluations.

Each subject was ophthalmologically investigated, with all routine tests performed. All subjects were asymptomatic at normal eye examination. ERG recordings were obtained on both eyes.

Inclusion criteria were: age between 20 and 80 years old and the absence of ophthalmological, neurological or vascular disabilities that might taint the ERG.

Exclusion criteria were: subjects older than 80 years or younger than 20 years, patients with ophthalmological, vascular or neurological disease, or patients on long term medications.

We recorded the ERG at Ophthalmological Research Centre "Ocularius", Craiova, under an agreement with the University of Medicine and Pharmacy of Craiova. We used the MonPackOne System (Metrovision, Perenchies, France). Recording electrodes were HK loop type ("Hawlina- Konec loop") and the reference and ground ones were Ag-AgCl cup type.

The study was carried out in compliance with the Declaration of Helsinki for the use of humans in research. Each subject was given detailed information about the study and signed an informed consent form.

Recording protocol

The research methodology consisted, for each subject, in:

A. Usual eye examination: visual acuity, biomicroscopy, intraocular pressure, fundus examination, refraction, color vision,

B. ERG recording according to ISCEV protocol:

1. fully dilatated pupils using 1% tropicamide and 2.5% phenylephrine eye drops;

2. skin cleansing with an abrasive paste and medicinal alcohol;

3. 4% xiline in the lower conjunctival bag;

4. electrods' placement: the active electrodes were placed on the free edge of the lower eyelid for each eye; the reference electrodes were placed near each orbital rim, with the ground electrode placed on the vertex, using a conductive paste;

5. dark adaptation for 20 minutes;

6. dark adapted ERG recording:

- rod response: the stimulus is a dim blue flash of 0.01 $cd.s.m^{-2}$, with an interval of 2 seconds between flashes;

- combined rod-cone response: the stimulus is a white flash of 3.0 $cd.s.m^{-2}$, with an interval of 10 seconds between flashes;

- oscillatory potentials: the stimulus is a white flash of 2.0 $cd.s.m^{-2}$, with an interval of 165 seconds between flashes.

7. light adaptation for 10 minutes;

8. light-adapted ERG recording:

- single flash cone response: a 3.0 $cd.s.m^{-2}$ stimulus, with an interval of 0.5 seconds between flashes and a background luminance of $30cd.m^{-2}$

- 30 Hz flicker: 30 stimuli of $3.0cd.s.m^{-2}$ per second.

Statistical analysis

We used Microsoft Excel (Microsoft Corp., Redmond, WA, USA), together with the XLSTAT add-on for MS Excel (Addinsoft SARL, Paris, France) and IBM SPSS Statistics 20.0 (IBM Corporation, Armonk, NY, USA) for processing the data.

To test data for normality, we used the Anderson-Darling test. Because almost all numerical variables investigated had a normal distribution of data, globally or inside each studied age group, we were allowed to use parametric statistical tests (e.g. ANOVA) and the results were summarized as mean value ± standard deviation.

In case the ANOVA test proved the existence of statistically significant differences, we used Fisher's LSD post-hoc test to determine the pairs of age groups for which the differences were significant.

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Results

At the end of the study, we had valid ERG recordings for all tests from 54 subjects, 30 women and 24 men; because there weren't any significant gender-related differences, we analyzed both genders together. Also, there were no significant differences between right and left eyes' values, so we decided to use the average values for each individual.

Our group was divided according to the subjects' age, resulting in 5 sub-groups with the following structure (Table 1):

Table 1. Subjects' distribution by age group

Age group	**19-29 years**	**30-39 years**	**40-49 years**	**50-59 years**	**>60 years**
No. of recordings	13	11	13	9	8

Average amplitudes, especially for b waves, were greater in younger subjects than in older subjects. Because of increased variability, we could not prove that observed differences for amplitudes held statistical significance. As such, we present in detail only the results concerning the implicit times.

Analyzing latency values recorded for standard tests (Table 2), we found statistically significant and highly significant differences between the five investigated age groups for the following items:

-a and b wave recorded for dark-adapted 0.01 ERG (Fig.1)

-a and b wave recorded for dark-adapted 3.0 ERG (Fig.2)

-b wave recorded for light-adapted 3.0 ERG (Fig.3)

-b wave recorded for light-adapted flicker 3.0 ERG (Fig.3)

Table 2. Implicit time results for the investigated ERG recordings (mean ± SD). Significant results are underlined

Age (years)	**Sc 0.01 ERG a**	**Sc 0.01 ERG b**	**Sc 3.0 ERG a**	**Sc 3.0 ERG b**	**Sc 3.0 OP 1**	**Sc 3.0 OP 2**	**Sc 3.0 OP 3**	**Sc 3.0 OP 4**	**Ph 3.0 ERG a**	**Ph 3.0 ERG b**	**Ph 3.0 Flicker a**	**Ph 3.0 Flicker b**
19-29 years	26.58 ± 2.57	52.73 ± 3.44	16.03 ± 1.76	37.34 ± 2.96	21.27 ± 0.40	24.77 ± 0.44	28.29 ± 0.75	31.85 ± 1.28	15.22 ± 0.66	30.82 ± 0.92	17.83 ± 1.79	28.63 ± 1.12
30-39 years	26.70 ± 3.17	53.83 ± 2.44	16.93 ± 2.47	40.61 ± 2.51	21.56 ± 0.63	25.05 ± 0.68	28.45 ± 0.60	31.77 ± 0.72	15.16 ± 0.59	31.05 ± 1.01	16.78 ± 1.74	29.44 ± 1.55
40-49 years	28.70 ± 2.56	57.03 ± 3.91	18.05 ± 2.58	38.57 ± 1.70	21.61 ± 0.57	25.16 ± 0.61	28.59 ± 0.68	32.60 ± 1.66	15.52 ± 0.54	31.03 ± 1.03	17.88 ± 1.73	29.44 ± 1.31
50-59 years	29.51 ± 1.90	60.79 ± 4.64	19.98 ± 2.44	41.54 ± 2.54	21.96 ± 0.68	25.46 ± 0.80	28.80 ± 0.95	32.27 ± 1.07	15.74 ± 0.75	30.66 ± 1.05	18.41 ± 1.58	30.15 ± 2.54
>60 years	29.72 ± 2.77	61.41 ± 3.56	22.08 ± 2.06	44.23 ± 6.16	21.85 ± 1.09	25.53 ± 1.30	29.25 ± 1.82	33.24 ± 2.38	16.01 ± 1.42	32.58 ± 1.56	18.35 ± 2.61	31.23 ± 2.67
p ANOVA	**0.024**	**< 0.0001**	**< 0.0001**	**0.000**	0.179	0.183	0.292	0.227	0.144	**0.010**	0.339	**0.047**
Signif.	Yes, S	Yes, HS	Yes, HS	Yes, HS	No	No	No	No	No	Yes, S	No	Yes, S

For the statistically significant results, we continued the analysis with Fisher LSD post-hoc tests, and the following differences between age groups were highlighted:

-For dark-adapted 0.01 ERG a wave: 19-29 years vs. all groups above 40 years, 30-39 vs. 50-59 and older

-For dark-adapted 0.01 ERG b wave: all pairs of groups, excepting 19-29 vs. 30-39 and 50-59 vs. > 60 years

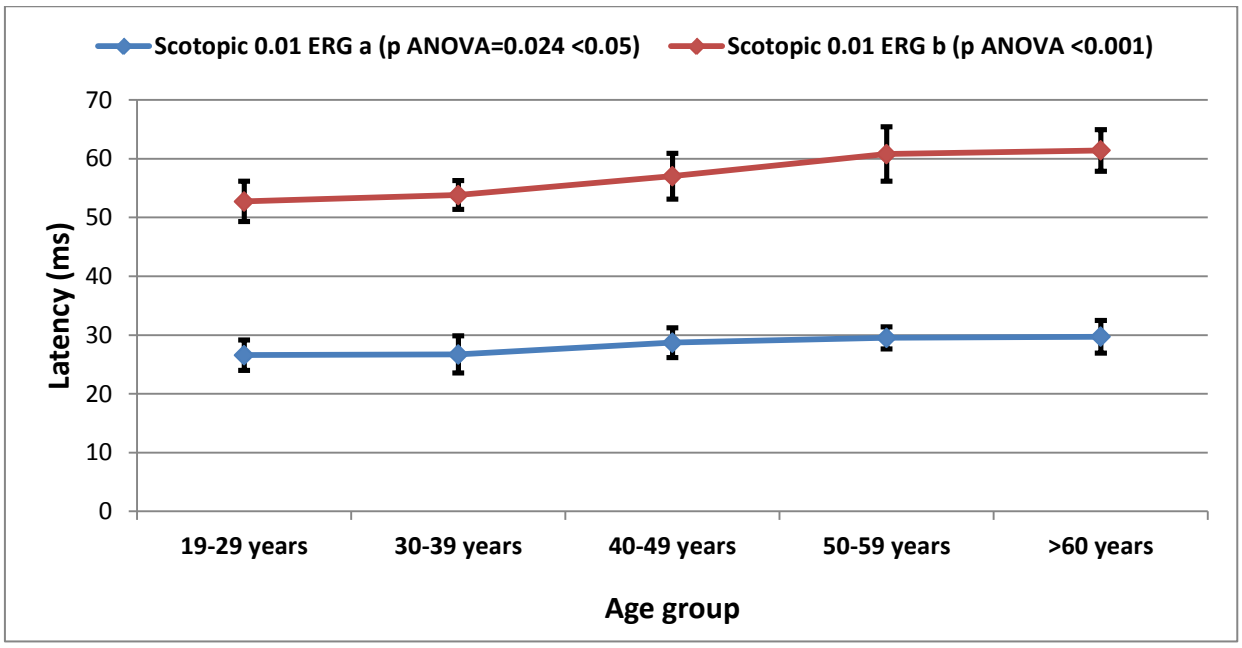

Fig.1. Comparison of a and b wave implicit times for dark-adapted 0.01 ERG

-For dark-adapted 3.0 ERG a wave: only the pairs that were one after the other did not show significant differences (e.g. 30-39 vs. 40-49)

-For dark-adapted 3.0 ERG b wave: >60 years vs. 19-29, 30-39, 40-49 years, and 19-29 vs. 50-59 years

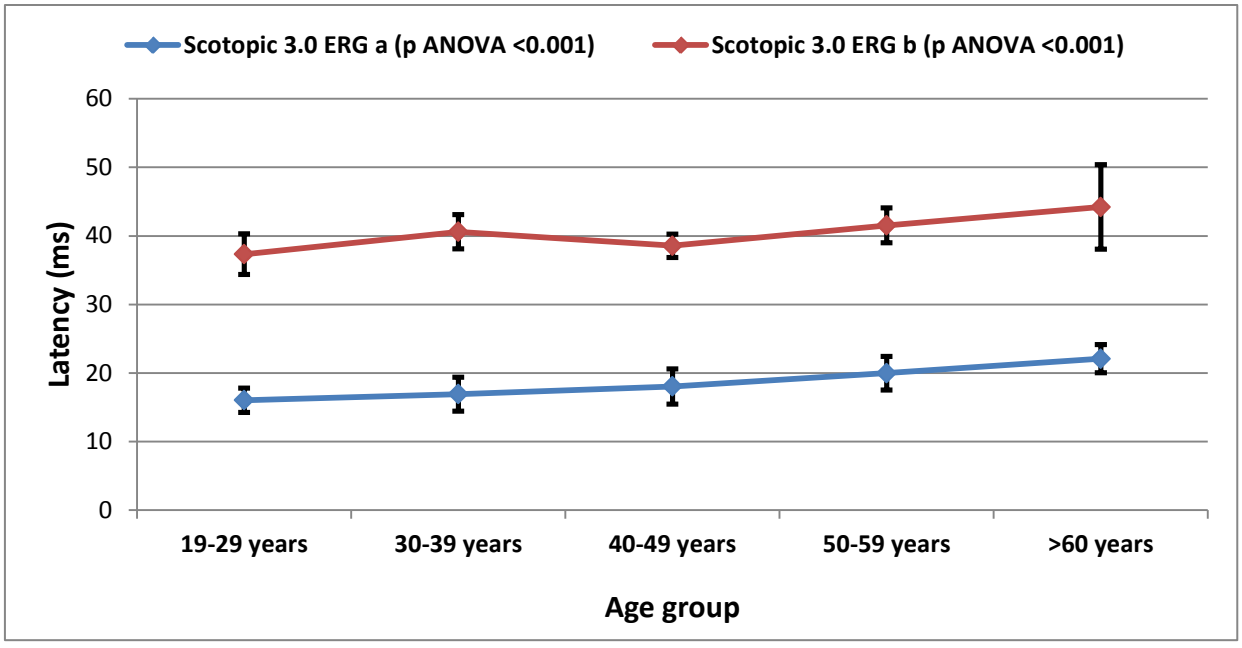

Fig.2. Comparison of a and b wave implicit times for dark-adapted 3.0 ERG

-For light-adapted 3.0 ERG b wave: 19-29 years vs. 50-59 and older

-For light-adapted flicker 3.0 ERG b wave: 19-29 years vs. 50-59 and older

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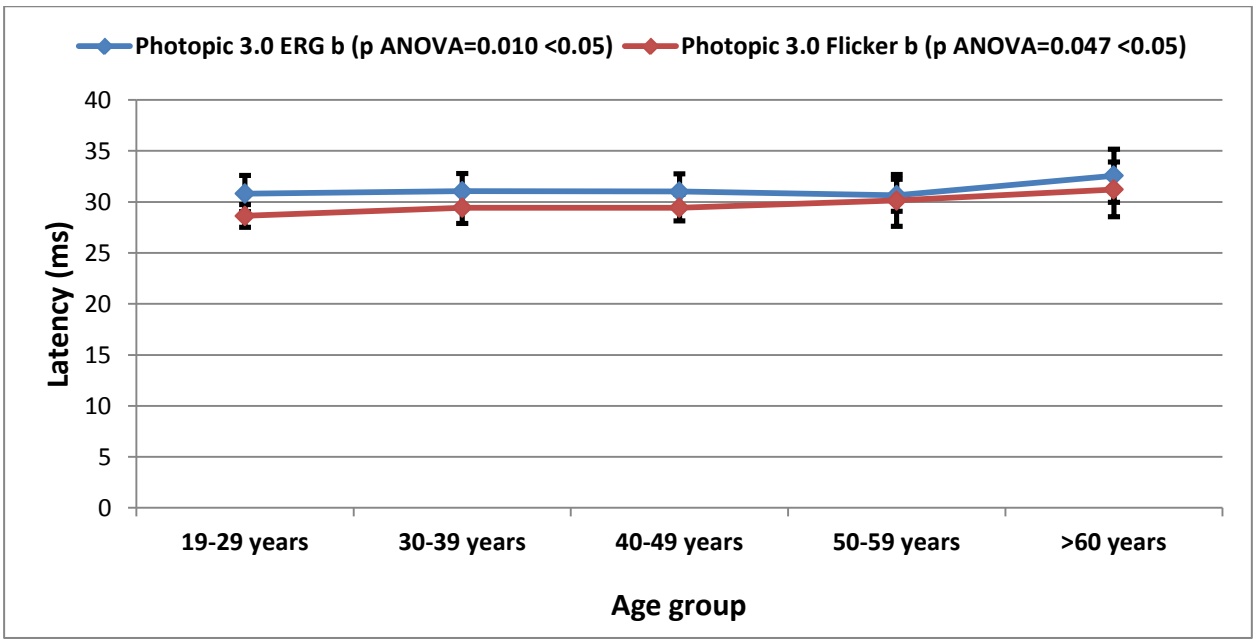

Fig.3. Comparison of b wave implicit times for light-adapted 3.0 ERG and light-adapted flicker 3.0 ERG

Discussions

The decrease of amplitude and increase of implicit time with age has been demonstrated for a long time [10,11] and documented ever since. Although, some aspects of normal ERG recordings still are under debate. For example, even if some classic ERG studies described gender-related differences of ERG parameter [10,11], (for example, women are supposed to have higher amplitudes for b waves elicited), our data do not support those results, information that is in agreement with other recently published studies, carried out on larger samples of normal subjects [12,13].

For the subjects investigated, we found the implicit times for dark-adapted waves to be lower than those reported on a study conducted on a population with different ethnicity [12], but they were similar to classical studies [10], studies strictly comparing Caucasian young and old subjects [13], or works reporting normal ERG values [14], while the light adapted implicit times had similar values in all studies. The difference between age groups proved to be more evident in our case; for our study the age-related delay of implicit time was significant a decade earlier than in other studies [12, 13].

The fact that the scotopic response has both a and b waves modified and delayed, while the photopic response shows only a modified b wave, leads to the conclusion that rod activity declines with age at a steeper rate than cone activity.

As it has been already described, the physiological variability of a and b wave amplitudes in healthy subjects [10,12-15], further amplified by recording conditions variability (i.e. electrode impedance), lead to the impossibility to prove there are statistical significant differences among amplitudes recorded for different age groups, in our case. Even if the general trend showed a decrease of amplitudes with age, especially for b waves, none of the statistical tests' results resulted in p values <0.05, as opposed to results of similar studies [10,12,13]. This shortcoming might be addresses by expanding our study to include a larger number of subjects, especially for the 50-59 and >60 years old groups.

Conclusions

This study proves there is a major loss in retinal activity due to aging, most of it being caused by the rod cells altered response, modifications that can be significant for the target population of our study, starting as early as from 50 years of age.

Also, oscillatory potentials do not seem to be much affected by age, and could prove a valuable test to investigate for changes in patients with Diabetes mellitus.

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Corresponding Author: Dragoş O. Alexandru, M.D., Ph.D., University of Medicine and Pharmacy of Craiova, Petru Rares St., No.2, Craiova-200349, Romania; e-mail: dragos.alexandru@umfcv.ro

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