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The effect of bleach duration and age on the ERG photostress test

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Key words: Electroretinogram, ERG; cone adaptation; repeatability; age-related macular degeneration

Abstract

Background: The ERG photostress test assesses the recovery of the focal 41Hz ERG following exposure to a bright light that bleaches a significant proportion of photopigment. The aims of this study were 1) to compare the repeatability of the ERG photostress test recovery time constant following long and short duration light exposure, and 2) to determine the effect of age on the ERG photostress test recovery time constant.

Methods: Focal 41 Hz ERGs were recorded from 23 participants (age range 20-71 years) at 20 second intervals for 5 minutes following either a short duration (photoflash) or long duration (equilibrium) light exposure. After a 5 minute wash-out period, the procedure was repeated using the second bleach modality. The time constant of cone recovery was determined by fitting an exponential model to the amplitude recovery data. The whole procedure was repeated on a second occasion. The co-efficient of repeatability (CoR) was calculated for each bleaching technique. The relationship between the time constant of recovery and age was investigated (Pearson’s correlation coefficient).

Results: The time constant of recovery following an equilibrium bleach was more repeatable than recovery following a photoflash (CoR = 85s and 184s respectively). Eight trials (from 7 participants) failed to show a reduction in amplitude following the photoflash, suggesting that a blink or fixation loss had occurred. All participants were reliably light adapted by the equilibrium bleach. For the equilibrium bleach data, the time constant of recovery increased with age at a rate of 27 seconds per decade.

Conclusions: The equilibrium bleach was more reliable and repeatable than the photoflash. Increasing participant age was shown to result in a lengthening of the recovery time constant of a magnitude comparable to previously published psychophysical data.

Introduction

The electroretinogram (ERG) photostress test [1-2] was developed as a dynamic test of outer retinal function, which assesses the recovery of the 41 Hz ERG amplitude, following exposure to a bright light. The technique is capable of differentiating patients with early age-related maculopathy (ARM) from healthy controls, even when VA remains near normal [2].

In dark adaptation and photostress studies, photopigment bleaching has been elicited in one of two ways. The first method typically involves the use of a photoflash unit which produces a very brief but intense flash of light [3-8]. The second method is to expose the retina to a less intense but longer duration light until equilibrium is reached between photopigment bleaching and regeneration [1-2, 9-11]. The literature suggests that the kinetics of cone photopigment recovery are different in these two situations. Following an equilibrium bleach the time constant of cone recovery is not dependent on the percentage of photopigment bleached, whilst the recovery following a photoflash is prolonged with an increasing percentage of photopigment bleached [3]. For exposures that bleach the same amount of photopigment, the time constant of cone recovery is also shorter after exposure to a brief flash than after an equilibrium bleach [3]. Given that clinic time is at a premium, the use of a photoflash is an attractive alternative to the longer duration equilibrium bleach currently used with the ERG photostress test [2].

To investigate the potential utility of this approach the first aim of this study was to compare the inter-session repeatability of ERG photostress test results obtained with equilibrium and photoflash bleaches. The psychophysically determined time constant of cone dark adaptation is known to be affected by age [4], with a reported increase of 0.21 minutes per decade. Therefore the second aim of this study was to investigate the effect of age on the time constant of cone recovery as determined by the ERG photostress test with both bleaching techniques.

Material and methods

Participants

Twenty-three healthy participants, aged 20 to 71 years (mean age 43.5±17.6 years) were recruited for this study from staff, students and volunteers attending the Eye Clinic at the School of Optometry and Vision Sciences. All participants had a corrected visual acuity of 0.2 (LogMAR) or better, clear ocular media, normal retinal / optic disc appearance, no history of retinal or systemic disease, and were not taking medication known to affect retinal function. The study adhered to the Tenets of the Declaration of Helsinki and was approved by the School of Optometry and Vision Science Research Ethics Committee. Each participant was given a full explanation of the procedures involved and their written informed consent was obtained before participation in the study.

ERG Recording

The earth electrode was a silver-silver chloride touchproof skin electrode (Viasys Healthcare Ltd., Warwick, UK) applied to the midfrontal position, whilst active and contralateral reference electrodes consisted of DTL fibres (Unimed Electrode Supplies, Surrey, UK) positioned in the lower fornix of both eyes. Contralateral corneal reference electrodes have been reported to provide larger ERG amplitude responses than traditional skin electrodes [5].

ERGs were recorded in response to a focal (20° diameter) amber stimulus (peak output 595nm, half height band width 17 nm) produced by a miniature Ganzfeld LED stimulator (CH electronics, Kent, UK). The stimulator comprised an array of LEDs set behind a circular diffuser. The LEDs provided a square wave flicker stimulus at a frequency of 41 Hz (50% duty cycle, flash duration 12 ms) with a time-averaged luminance of 30 cd/m^2. The stimulus subtended 20° at the eye, when viewed from a distance of 14 cm, and was set within a luminance-matched Ganzfeld surround to suppress responses from the peripheral retina. Stimulus luminance was measured with a photometer (LS-110; Konica Minolta, Osaka, Japan).

An evoked potential monitoring system (Medelec Synergy EP; Oxford Instruments Medical, Surrey, UK) was used to record all ERGs in the course of this study. ERG responses were recorded monocularly, bandpass filtered (1 to 100 Hz) and digitally averaged. A 50 ms time base at a sampling rate of 20 KHz was used with one hundred sweeps (each consisting of two response cycles) averaged per trace. An artefact reject setting (50 µV) allowed the exclusion of traces contaminated by eye movements.

Bleaching Techniques

The long duration ‘equilibrium’ bleach was provided by a tungsten halogen source which was presented to the subject within a ganzfeld bowl. A central fixation cross was placed within the ganzfeld bowl such that the bleaching source subtended 40° at the eye. The flickering amber stimulus was placed directly above this, allowing the subject to take up position for ERG recording quickly at cessation of the photobleach. Heat filters were in place, which reduced output of the bleaching light to below 5% between 800-900nm so that excessive infra-red (IR) radiation did not reach the eye.

Using this apparatus the eye was light-adapted to a bright white background of 5.6 log td for a period of 2 minutes. The effective retinal illuminance was calculated as 5.2 log photopic td, when adjusted for the Stiles-Crawford effect, which bleached approximately 84% of the cone visual pigment [3].

The short duration “photoflash” bleach was provided by a Metz Mecablitz 76 MZ-5 flashgun (Metz-Werke GmbH & Co., Zirndorf, Germany), positioned such that this source also subtended 40° at the eye when centrally fixated. The eye was exposed to a bright white flash of 7.3 log td.s for a period of approximately 6.6 ms. The effective retinal illuminance, adjusted for the Stiles-Crawford effect, was calculated as 6.9 log photopic td.s, which bleached approximately 98% of the cone visual pigment [6-7].

The time constant of cone recovery following an equilibrium bleach is independent of the percentage bleach achieved [3]. However following a ‘non-equilibrium’ short duration bleach the time constant is shorter [3, 8], and is variable, with the quickest recoveries following less intense bleaches [3]. Therefore, the maximum intensity bleach from the flash unit was used in this study, theoretically producing the longest recovery times over which to monitor the ERG recovery.

Heat filters were used to attenuate output to below 5% between 800-900nm, so that excessive infra-red (IR) radiation did not reach the eye. Additionally a UV filter integrated within the flash gun eliminated wavelengths below 375 nm. All bleach luminance measurements were made using an IL1700 photometer (International Light Inc, Newburyport, MA) and exposures were within the safety guidelines set out within BS EN 15004-2 [9].

General procedure

Both bleaching protocols were evaluated at the same recording session and participants were randomly assigned to one of two groups determining whether the photoflash or equilibrium bleach was to be used first. The eye with better visual acuity was chosen for testing, with the left eye chosen as default in cases of equal acuity. The non test-eye was patched. Pupils were dilated with 1 drop of 1.0 % Tropicamide prior to electrode attachment.

Prior to recording, a 5 minute period of adaptation to the flickering stimulus and surround was undertaken to avoid any flicker adaptation effects during the recording period. In order to prevent any residual bleach effect between tests, a 5 minute break was implemented. Initially four 41Hz flicker ERG traces, each consisting of the average of 100 sweeps, were recorded to provide a baseline pre-bleach amplitude. This was followed by exposure of the eye to either the photoflash or equilibrium bleach. Upon completion of the bleach, the participant had 10 seconds to align themselves with the stimulus for the recording of the first trace; a total of 15 traces were then recorded at 20 second intervals over a 5 minute period. Each trace took approximately 5 seconds to record, however, the time required was extended if any blinks or other contamination occurred which led to trace rejection. This would normally leave 10 to 15 seconds between successive recordings for the participant to relax and blink. The entire protocol was repeated on a second occasion within 4 weeks of the first visit for every study participant. A sequential representation of the protocol undertaken at each visit is shown in Figure 1.

Figure 1 about here

Analysis

The amplitude of the fundamental frequency of each trace was determined by Fourier analysis using Excel 2003 (Microsoft. Redmond, WA) and plotted as a function of time after cessation of the bleach. In order to limit the effect of any noise or contamination of individual traces, especially in those traces immediately post-bleach where the signal-to-noise ratio was low, the Fourier analysis for all post bleach traces was phase locked to the mean of the 4 pre-bleach traces. In this way only 41 Hz signals which were the same phase as the pre-bleach signal were extracted.

The time constant of cone photopigment regeneration was determined by fitting Equation 1 to the amplitude recovery data on a least squares fit basis using the Solver function of Excel 2003 (Microsoft. Redmond, WA).

Equation 1: $\textbf{Amplitude (t) = a [1-B*exp (-t/}\boldsymbol{\tau}\textbf{)]}$

Where “t” is time after the photobleach in seconds, “a” is the pre-bleach amplitude, “B” is the proportion of photopigment bleached (where B=0 signifies 0% and B=1 signifies 100%) and “τ” is the time constant in seconds. “B” was constrained within the model to return a value of 0 or greater. Inter-session repeatability of the time constant of recovery was assessed by calculating the coefficient of repeatability (CoR) (determined as 1.96 x the standard deviation of differences between visits 1 and 2). The repeatability was also graphically demonstrated by plotting the difference in time constant between visits 1 and 2 against the mean time constant for both visits, a technique advocated by Bland and Altman [10]. The agreement between recovery time constants for long and short duration bleaches was also presented using this [Bland and Altman] approach [10].

The effect of age on the recovery time constant was also assessed. Recovery time constants for all participants were plotted as a function of age, with the gradient of the best fitting line indicating the change in time constant per decade of life. Pearson’s correlation coefficient was calculated to determine whether this relationship was significant.

Results

Typical 41 Hz ERG traces for 3 participants aged 23, 44 and 60 are shown in Figure 2. The initial 4 traces shown are the pre-bleach baseline and represent the expected waveform after a full recovery. The subsequent 15 traces were recorded at 20 second intervals following the bleach. The recovery in ERG amplitude post-bleach, towards the baseline level, is apparent in these participants for both bleaching modalities. The amplitude values plotted against time for these 3 participants are shown in Figure 3. The amplitude recovery has been fitted with Equation 1.

Figures 2 and 3 about here

For the group of 23 participants, a total of 46 ERG photostress tests were recorded using the equilibrium bleach and 46 were recorded using the photoflash bleach. From the trials conducted using the equilibrium bleach, only 1 was excluded due to excessive recording noise; however, of the trials conducted using the photoflash, 8 were excluded due to ineffective bleaching i.e. the ‘bleach’ did not diminish the amplitude of the 41 Hz ERG and hence there was no recovery. Of the 8 failed photoflash bleaches, one participant did not produce valid results on either visit. The mean time constants for the equilibrium (117 ±72s) and photoflash bleach (112 ±58s) techniques were not significantly different (paired t-test; P=0.992).

Figure 4 describes the repeatability of each technique, the CoR was 85s (n=22) for the equilibrium bleach and 184s (n=16) for the photoflash bleach. The mean difference between visits was close to zero, indicating no bias between visits (see Figure 4).

Figure 4 about here

Figure 5 compares the mean time constant of recovery between the two techniques for the 15 participants where successful bleaches were achieved for both bleach techniques. There was no bias towards a longer time constant for either technique.

Figure 5 about here

Figure 6 plots the time constant of recovery as a function of age for both techniques. For the equilibrium bleach data, the time constant (τ) of recovery increased by 27.6s per decade of life, this relationship was statistically significant (Pearson's correlation coefficient r=0.66, P=0.0008). There was also a subjectively evident increase in variability with older subjects. There was no significant relationship between age and time constant of recovery for the photoflash technique (Pearson's correlation coefficient, P=0.19).

Figure 6 about here

Discussion

The results provide compelling evidence for the retention of the equilibrium bleach as part of the ERG photostress test. The equilibrium bleach showed relatively good repeatability (CoR 85s) and was successfully recorded on 45 out of 46 occasions. The photoflash bleach, by comparison, was less repeatable (CoR 184s) and did not always provide an effective bleach.

Previously we have shown that the ERG photostress test can differentiate those with early ARM from age matched controls, demonstrating a mean difference of 106s in time constants between groups [2]. Given that the coefficient of repeatability for the photoflash technique (184s) is larger than the difference between those with and without disease (106s) it is apparent that the sensitivity and specificity of the ERG photostress test would be compromised by switching to the photoflash bleaching method.

It is noteworthy that this assessment of the repeatability of the photoflash bleaching method only included data from 16 out of 23 subjects for whom a post bleach recovery was available. The observation that the photoflash unit did not diminish the amplitude of the 41Hz ERG in 7 participants suggests that blinks or gross fixation losses must have coincided with the timing of the photoflash discharge. Given the number of bleach failures additional bleaches would need to be administered to obtain satisfactory results. In a clinical situation this would increase examination time making the technique clinically nonviable. The instantaneous nature of the photoflash exposure may also have increased the potential for partial bleaches due to inaccurate patient fixation and incomplete blinks, and this may have contributed to the relatively poor CoR of this technique.

In contrast, the equilibrium bleach allows 2 minutes to bleach the retina, therefore transient fixation losses and blinking are unlikely to affect the photopigment bleach obtained. In addition, there is a theoretical basis for assuming that an equilibrium bleach may provide a better separation between individuals with ARM and age matched controls than a photoflash bleach. The rod photoreceptors, when bleached, obtain the retinal required to regenerate photopigment from the RPE. As a result, the rate of photopigment regeneration within the rods is dependent on the health and function of the RPE and the diffusion of retinoids to the RPE from the choroidal circulation via Bruch's membrane [11]. The cone photoreceptors, however, are able to regenerate photopigment using a local store of retinoid derived from the Müller cells [12], and therefore do not necessarily have the same dependence on the health of the RPE, Bruch's membrane and choroidal circulation. Abnormal RPE/Bruch's function may have little or no effect on cone photopigment regeneration whilst this local retinoid store is present. Unlike photoflash bleaches, long duration bleaches are likely to deplete local stores of 11-cis-retinal [8], placing greater emphasis on the role of the RPE in cone photopigment regeneration. Hence long, but not short, duration bleaches may help elucidate functional delays in people with ARM.

It has been reported that the rate of dark adaptation decreases as we get older [13]. The effect of age on recovery time constant in this study produced an increase of 27 seconds per decade (0.45 minutes / decade) which was comparable to the findings of Coile & Baker [4] who showed an increase of 0.21 minutes per decade. Although the relationship between age and time constants in this study can be fitted using a single linear function, it is possible that a steeper rate of change above 55 years may be present as suggested by Newsome et al [14]. The increased variability in time constant with age makes this difficult to determine.

In agreement with our findings, Coile & Baker [4] also demonstrated a greater variability in the rate of adaptation of older participants compared to younger ones. The increased variability could suggest that those apparently normal individuals with prolonged time constants may be at risk of developing ARM. Currently ARM is identified on the basis of fundus photography, but it is possible that significant changes occur in the function of the retina prior to the macroscopic changes, such as drusen and pigmentary alteration, becoming visible on ophthalmoscopic examination. Histological work by Sarks [15] identified changes to Bruch's membrane and the presence of basal linear deposits within the retina in eyes with a normal fundus appearance and often good visual acuity. Changes in the permeability of Bruch's membrane have been suggested to be the cause of delayed dark adaptation in ARM [16], so individuals with pre-clinical ARM might be expected to show abnormal time constants. Confounders such as media opacities could also be influencing the spread of data. The increase in lenticular changes with age would conceivably lead to more variability in retinal illumination, and therefore increase the variability of the results. However, this is unlikely as all those with marked lens opacities were excluded. Hollins and Alpern [3] showed that equilibrium bleaches at a range of intensities all produced the same time constants of recovery, making any difference in τ due to minor media opacities unlikely.

The photoflash data demonstrated a similar time constant to the equilibrium bleach technique. A shorter recovery may have been expected following the photoflash had a similar proportion of photopigment been bleached for each technique [7], however given the higher percentage bleach provided by the photoflash, the lack of a significant difference in recovery time is not unexpected. Additionally the photoflash technique did not demonstrate a significant relationship with age (see Figure 6). This was attributed to the variability of the post photoflash ERG data, particularly the traces recorded immediately post bleach. Fitting equation 1 to noisy data will inevitably result in aberrant recovery times. This corresponds to participant reported, and observed, difficulty in avoiding blinks and eye movements for the initial ERG recordings after a photoflash, an observation not apparent following the equilibrium bleach.

Many studies have successfully used photoflash bleaches [17-18], some of which report using techniques to hide or obscure the flash source [14, 19], potentially improving reliability of flash delivery by reducing anticipation. Most importantly these studies assessed psychophysical responses, where the effects of blinking and eye movements following the flash exposure are likely to have a minimal impact on data quality and reliability compared to the electrophysiological techniques used in this study.

In conclusion, the established equilibrium bleach technique was found to give good intersession repeatability, sufficient to be sensitive to ARM related changes. The alternative photoflash bleaching technique was less repeatable and clinically unreliable. Finally the time constant of recovery as determined using the equilibrium bleach was found to increase with age.

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Figure Legends

Figure 1: The timeline showing the recording process for the ERG photostress tests. The bleach type for tests 1 and 2 were randomly selected prior to recording for each participant, and bleach duration was either 6.6 ms or 2 minutes.

Figure 2: Raw 41 Hz ERG traces for participants a (aged 23 years), b (aged 44 years) and c (aged 60 years). Top panel shows pre-bleach traces, middle panel shows traces following an equilibrium bleach and the bottom panel shows the traces following a photoflash bleach.

Figure 3: ERG amplitudes for participants a, b and c plotted against time post bleach, and best fitting exponential model (Equation1) to recovery data following the equilibrium bleach (Participant a; τ = 95.6, Participant b; τ = 103.5,Participant c; τ= 157.8) and photoflash bleach (Participant a; τ = 77.8, Participant b; τ = 273.2, Participant c; τ = 127.5,).

Figure 4: Intersession difference plotted against intersession average providing a graphical representation of intersession repeatability as advocated by Bland and Altman [10]. The solid horizontal line represents the mean difference between visits 1&2, whilst the dotted lines indicate the 95% limits of agreement; a narrower interval between these lines indicates better repeatability. The coefficients of repeatability (1.96 x SD of differences) for each technique were 85s (equilibrium) and 184s (photoflash). This does not include data from those participants who were excluded due to ineffective bleaching.

Figure 5: Between technique differences in recovery time (mean short – mean long duration bleach) plotted as a function of average recovery time. The solid horizontal line represents the mean difference in recovery time constant whilst the dotted lines indicate the 95% limits of agreement.

Figure 6: The relationship between mean time constants (Visits 1 + 2) and age for equilibrium and photoflash bleaches. A statistically significant correlation was identified for the equilibrium bleach (Pearson’s correlation r=0.66 P=0.0008) whereas a non-significant negative correlation was found with the photoflash bleach (Pearson’s correlation r=-0.34 P=0.19). This does not include data from those participants who were excluded due to ineffective bleaching.

Figures

Figure 1.

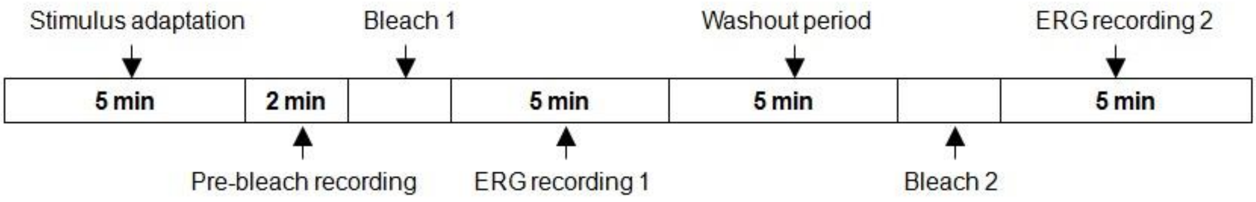

Figure 2.

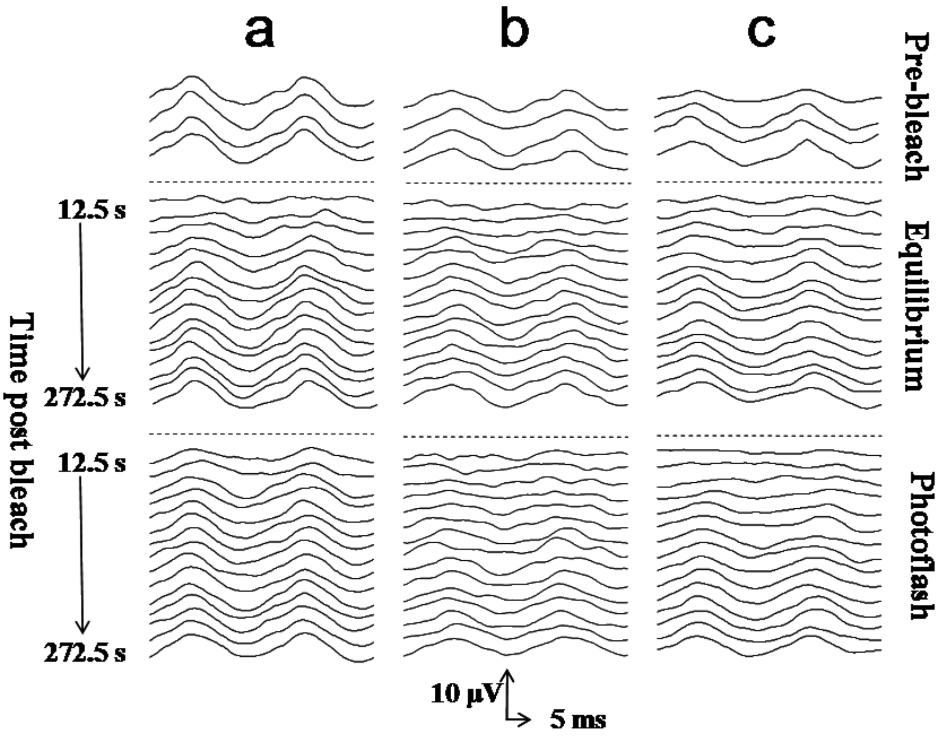

Figure 3.

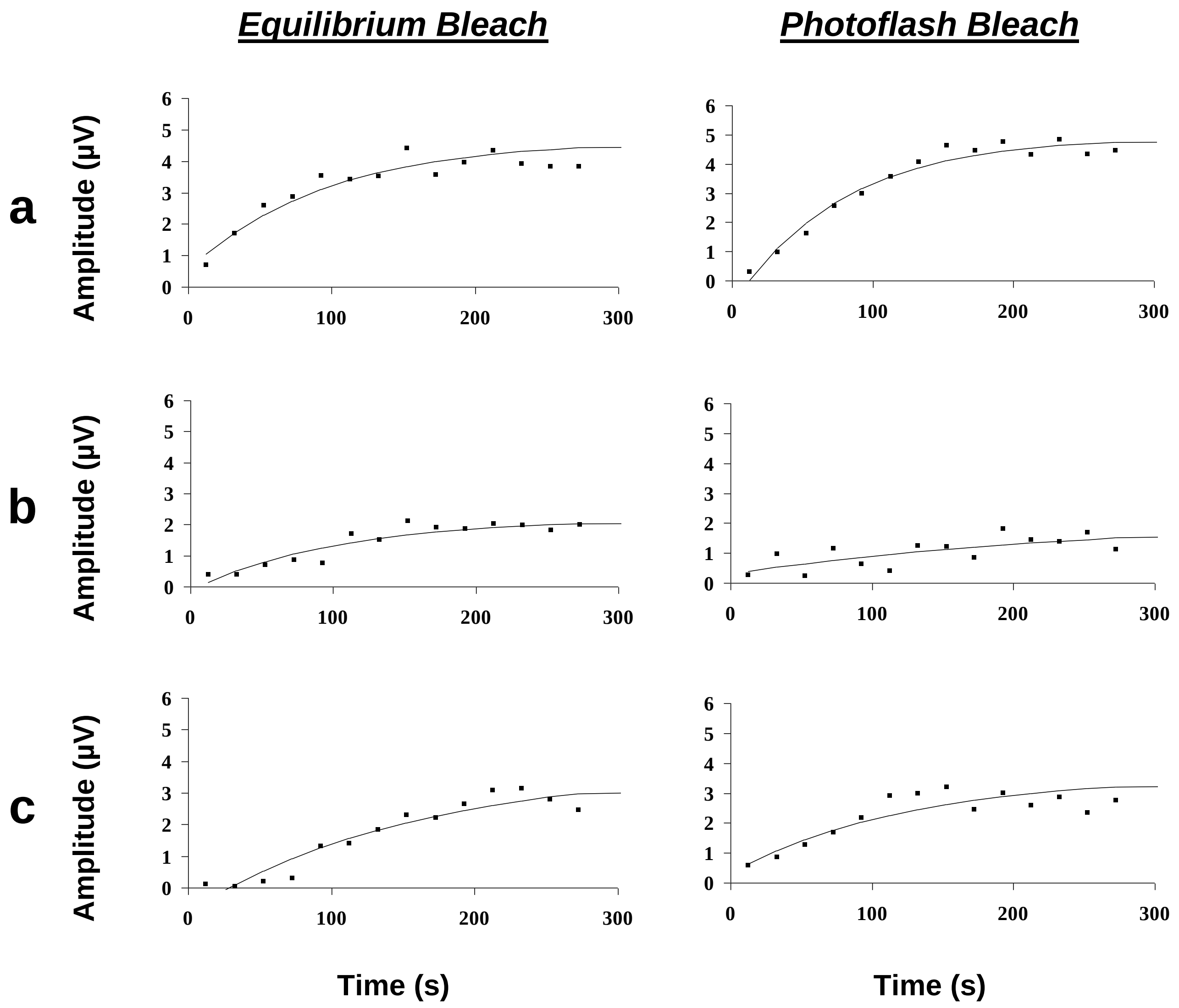
Equilibrium Bleach
Photoflash Bleach
a
b
c
Amplitude (µV)
Amplitude (µV)
Amplitude (µV)
0
1
2
3
4
5
6
0
100
200
300
Time (s)
Time (s)

Figure 4.

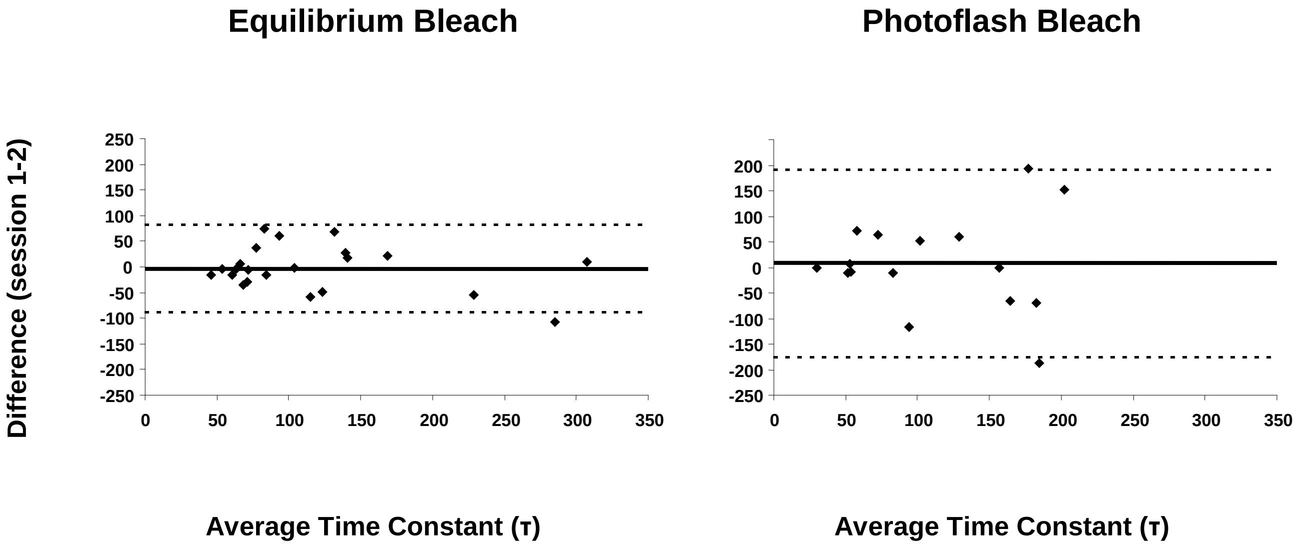
Equilibrium Bleach
Photoflash Bleach
Difference (session 1-2)
250
200
150
100
50
0
-50
-100
-150
-200
-250
0
50
100
150
200
250
300
350
Average Time Constant (τ)
Average Time Constant (τ)

Figure 5

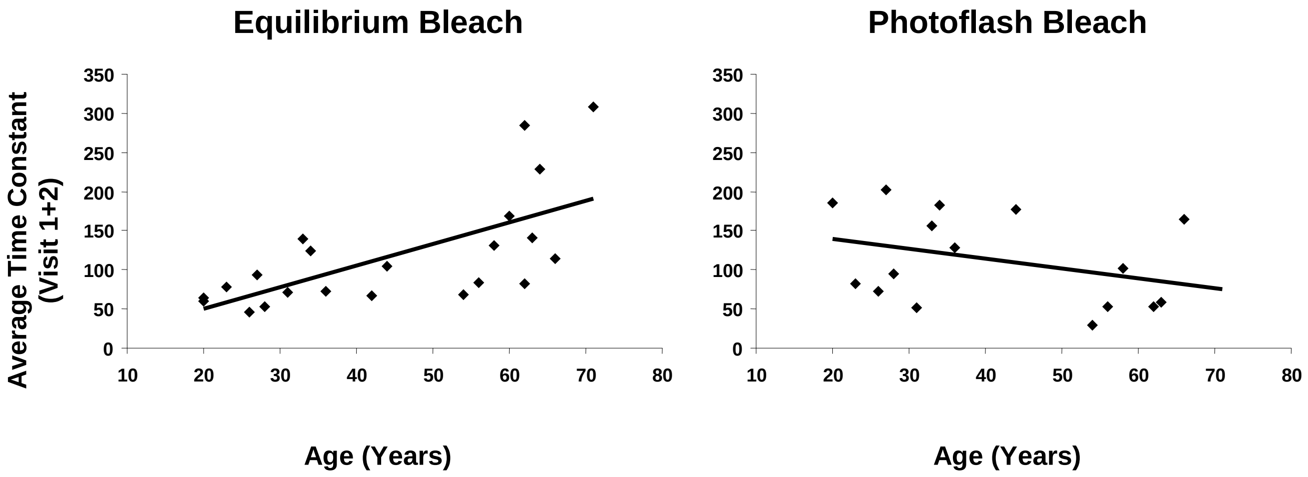
Equilibrium Bleach
Photoflash Bleach
Average Time Constant
(Visit 1+2)
350
300
250
200
150
100
50
0
10
20
30
40
50
60
70
80
Age (Years)
Age (Years)

Figure 6.