Clinical Neurophysiology 120 (2009) 1828–1834

Contents lists available at ScienceDirect

Clinical Neurophysiology

journal homepage: www.elsevier.com/locate/clinph

Lack of habituation in the light adapted flicker electroretinogram of normal subjects: A comparison with pattern electroretinogram

Antonello Fadda [a], Antonio Di Renzo [b], Vincenzo Parisi [b], Giovanna Stifano [c], Emilio Balestrazzi [c], Charles E. Riva [d], Benedetto Falsini [c,*]

[a] Istituto Superiore di Sanità, Dipartimento Tecnologie e Salute, Rome, Italy
[b] Fondazione Bietti-IRCCS, Rome, Italy
[c] Institute of Ophthalmology, Catholic University, Rome, Italy
[d] Department of Ophthalmology, University of Bologna, Bologna, Italy

ARTICLE INFO

Article history:
Accepted 18 June 2009
Available online 15 September 2009

Keywords:
Electroretinography
Flicker sensitivity
Inner retina
Habituation
Pattern stimulation

ABSTRACT

Objective: Sustained pattern stimulation (SPS) induces habituation in the normal pattern electroretinogram (PERG). In this study, the authors evaluated whether sustained flicker stimulation (SFS) induces habituation in the normal flicker ERG (FERG).

Methods: FERGs were elicited in normal volunteers by an 8 Hz flicker stimulus, presented continuously over 3 min after 20 min of light adaptation. One stimulus temporal period was sampled and averaged in packets ($n = 20$) of 60 events, each of 8 s duration. Amplitudes and phases of the response 1st and 2nd harmonics (1F and 2F, respectively) were measured. FERG results were compared with those obtained by recording PERGs with a similar SPS paradigm.

Results: During SFS, FERG 2F showed a modest increase in amplitude (about 25%, $p < 0.05$). No changes were observed for the 1F amplitude and for the phase of both components. In contrast, PERG amplitude showed SPS-induced habituation, described by an exponential decay with a time constant of ~20 s.

Conclusions: The normal FERG, unlike PERG, does not show habituation, suggesting that the adaptive changes of retinal neurons underlying FERG are different from those of PERG generators.

Significance: Our findings may have implications for diagnosis and/or pathophysiology of retinal disorders involving the inner retina.

1. Introduction

Recent work (Porciatti et al, 2005) has demonstrated that the response of the pattern electroretinogram (PERG), a signal of inner retinal origin (Maffei and Fiorentini, 1981; Baker et al., 1988) evoked by a sustained stimulation by patterned fields, shows an exponential decrease of its amplitude towards a plateau, the latter reached after about 110 s. This effect, known as "habituation" occurs immediately after the start of the stimulus and is independent of stimulus luminance. The decrease in the response during habituation amounts to about 30% of the initial value. The authors hypothesized that the plateau represents a dynamic equilibrium between retinal ganglion cell activity and the available energy supply (Porciatti et al., 2005). The occurrence of the habituation process suggests a deficit between the metabolic cost to sustain the activity of the retinal ganglion cells and the available energy supplied to the neurons. Whether this deficit is due to some limitation of the vascular system to increase its supply according to the metabolic cost is not yet known.

Many lines of evidence indicate that the 1st and 2nd harmonics (1F and 2F, respectively) of the flicker electroretinogram (FERG) reflect the activity of different post-photoreceptoral retinal layers (Baker et al., 1988; Kondo and Sieving, 2002). Pharmacological studies (Kondo and Sieving, 2001, 2002) indicate that the 1F component is shaped mainly by the activity of ON- and OFF-bipolar cells. Clinical results in humans (Porciatti and Falsini, 1993; Falsini et al., 1995) show that the 2F component may be selectively altered (i.e. with preserved 1F component) in diseases affecting primarily the inner retina, such as glaucoma, optic neuritis or optic nerve compression (Porciatti and Falsini, 1993; Porciatti et al., 1989) and appears to be highly sensitive to retinal vascular disorders, such as diabetic retinopathy (Ghirlanda et al., 1991) and occlusion of a branch of the central retinal artery or vein (Falsini et al., 1995). PERG and FERG probably originate from partly different retinal sources (Baker et al., 1988) and therefore their behavior in response to a sustained stimulus presentation may differ.

* Corresponding author. Address: Università Cattolica S. Cuore, Lgo F. Vito 1, 00198 Rome, Italy. Tel.: +39 06 30154929; fax: +39 06 3051274.
E-mail address: bfalsini@unicatt.it (B. Falsini).

1388-2457/$36.00 © 2009 Published by Elsevier Ireland Ltd. on behalf of International Federation of Clinical Neurophysiology.
doi:10.1016/j.clinph.2009.06.011

The present study was undertaken to evaluate whether, in analogy with the PERG, the normal, light-adapted FERG also demonstrate slow adaptive changes, such as habituation, during sustained flicker stimulation.

2. Methods

2.1. Subjects

Fourteen normal subjects (seven males and seven females, mean age 28, standard deviation, SD, ±7 years) participated in the study. These normal volunteers were free from ocular or systemic diseases, had normal corrected Snellen acuity of 20/20 or better and refractive errors within ±3 sph and ±1 cyl diopters. The study adhered to the Tenets of the Declaration of Helsinki and was approved by the Institutional Review Board.

2.2. Apparatus and procedure

The FERG was elicited by the LED-generated sinusoidal luminance modulation of a circular uniform field (18° in diameter, 40 cd/m^2 mean luminance, dominant wavelength: 630 nm), presented at the frequency of 8 Hz on the rear of a ganzfeld bowl (Fadda and Falsini, 1997). The latter was illuminated at the same mean luminance as the stimulus. In the main recording protocol that was employed in all subjects, a sustained flicker stimulus (SFS) was presented at a fixed modulation depth of 93.5%, as quantified by the Michelson luminance contrast formula: $100\% * (Lmax - Lmin)/(Lmax + Lmin)$, where *Lmax* and *Lmin* are maximum and minimum luminance, respectively.

To check the response repeatability over a short period of time, two subjects underwent an additional protocol of repeated SFS experiments. The same general conditions were applied, allowing for 1 min recovery interval between repetitions. A maximum of four repetitions of SFS were recorded, given the significant effort required by the subjects to perform the task.

A subgroup of six subjects also took part in another experimental session in which the responses to flicker and pattern reversal stimuli were compared under similar recording conditions (see below). These subjects were first exposed to the SFS following the general protocol. Ten minutes after the end of this experimental session, they were also exposed to a sustained pattern reversal stimulation (SPS) consisting of a sinusoidal grating of 1.7 cycles/° spatial frequency and 90% contrast (mean luminance: 35 cd/m^2), modulated in counterphase at 7.5 Hz (15 reversals/second). The PERG was continuously recorded for 3 min, with a protocol very similar to that employed for flicker.

The small differences in temporal frequency and modulation depth for the stimuli employed in the FERG and PERG recordings were dictated, and constrained, by the differences in the properties of the two stimulators employed to elicit the two responses.

FERGs (and PERGs) were recorded as previously published (Falsini et al., 2002; Salgarello et al., 2008). Briefly, signals were monocularly derived, with an inter-ocular reference, by means of Ag–AgCl superficial cup electrodes taped over the skin of the lower eyelids, amplified (gain of 100,000, 1–250 Hz bandwidth, 6 dB/octave slope), digitized (12 bit resolution, 2 kHz sampling rate, 100 μV AC range) and averaged. The averaging time (i.e. the sweep duration) was equal to the stimulus temporal period (125 ms). Single sweeps exceeding a voltage window of ±30 μV were rejected to minimize noise coming from blinks or eye movements. A discrete Fourier analysis was performed in order to isolate the FERG 1F and 2F harmonic components, and the PERG second harmonic (2P), whose peak-to-peak amplitudes (in μV) and phases (in degrees) were determined. Given the sampling rate of 2 kHz and

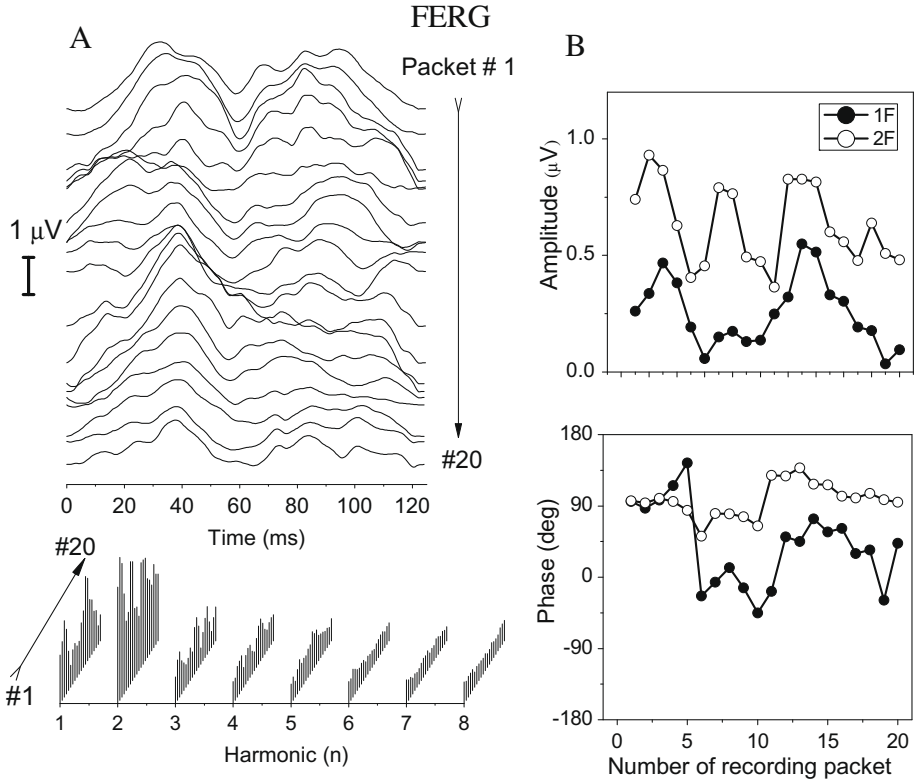

Fig. 1. (A) Representative examples of FERG recordings obtained by SFS in a subject during one experimental session. (Top) Individual packets as resulting from a smoothing procedure consisting of a three-point adjacent average. (Bottom) Fourier spectra (the first 8 harmonic components) of individual packets isolated by a discrete Fourier Series. (B) Plots of 1F and 2F amplitude and phase data as a function of the number of recording packet.

the sweep duration of 125 ms, the phase resolution of discrete Fourier Series was estimated to be at least 1.44° for the 1F component and 2.88° for the 2F. A second, asynchronous averaging channel, having a slightly detuned frequency (10% larger), was used to reject the signal and give an estimate of the background noise effects in the signal band, as it appears after the same processing (Salgarello et al., 2008). Resulting noise components amplitude ranged from 0.05 to 0.08 μV. In all subjects, both 1F and 2F, as well as 2P signal components were above the noise level (signal-to-noise ratio >2.8) and sufficiently reliable (i.e. the standard error, SE, of amplitude was typically less than 20% of the average amplitude, and the phase SE was within ±20°).

All subjects fixated at the center of the flicker stimulus. The pupils were not dilated. Their size was measured at the beginning of light adaptation (see also later), at the end of light adaptation, and just before the ERG recordings and at the end of the recordings.

2.3. FERG recording during SFS

The flicker stimulus presentation (approximately 3 min) was preceded by the adaptation to an unmodulated uniform field (approximately 20 min) kept at the same illuminance as the flicker mean illuminance. At the end of the adaptation period, a "noise" response was measured while the stimulus field was kept unmodulated. Noise recording consisted of 20 packets of 60 events each (160 s duration), with a protocol exactly identical to the ERG protocol (see below). Then the flicker stimulus was started and presented continuously over 160 ± 20 (SD) s. Twenty packets of 60 events each were collected during one recording session. The first five events were discarded to eliminate the transients at the onset of stimulation. Data collection and averaging of a single packet took an average of 8 s (±0.75 s, SD). Typically two recording sessions were obtained, with a between-session interval of 10 min for each experimental eye. The data were then exported to a text file and plotted as a function of packet number, which is directly related to time. If the amplitude and phase data of the two sessions were consistent (i.e. response amplitude change, expressed as the range of amplitude values across repetitions, within ±30%; phase changes <20°), they were averaged and used as a single entry for each subject. This was the case in about 90% of recordings. For the other 10% of the cases, additional recording sessions (1–3) were obtained. The results of the additional recording sessions were then compared to the grand average obtained in the same subject and subsequent sessions were added until it was verified that reproducibility criteria, expressed as standard error (SE) of mean amplitude (<20%) and of phase (<20°) were satisfied.

2.4. Statistical analysis

FERG 1F and 2F collected from the right eye of each subject first underwent a smoothing procedure, consisting in a three-point adjacent vector average (i.e. the averaging was performed on vector cartesian components and the results were expressed using polar representation, in terms of amplitude and phase). Circular standard deviations for the phase data were calculated according to Victor and Mast (1991). At the end of the procedure each recording session consisted of 20 packets (per component) characterized by amplitude and phase angle data. These packets were averaged across subjects and plotted as a function of packet number. The 1F and 2F amplitude data were also normalized by dividing each

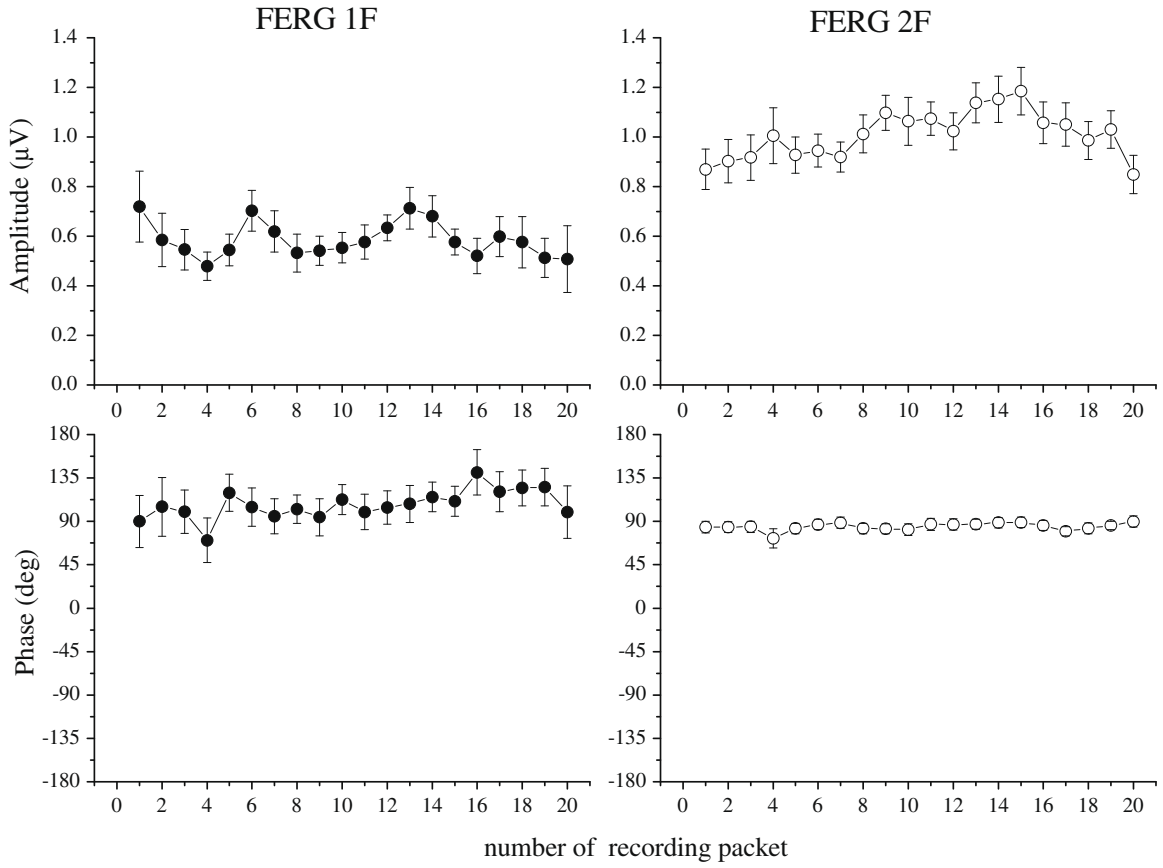

Fig. 2. Mean amplitudes and phases (±standard error, $N = 14$) of the FERG 1F and 2F components, recorded during sustained flicker stimulation, plotted as a function of the packet number. A packet represent 8 s of recording.

packet's amplitude by the mean amplitude calculated for every subject. This normalization minimized inter-individual variability while highlighting specific changes related to SFS. Based on the skewness and kurtosis results, the 14 individual 1F and 2F response amplitudes for every packet were normally distributed around the average, according to a Shapiro–Wilk test at a $p = 0.05$ significance level. Statistical analysis was therefore performed by a parametric repeated measures analysis of variance (ANOVA). Packet number was the within-subjects factor and component amplitude and phase angle were dependent variables. PERG amplitude data were fitted, both individually and as average value, by a single exponential decay function according to Porciatti et al. (2005). A p value <0.05 was considered as statistically significant.

3. Results

Throughout the different experimental sessions, no significant changes in mean pupil size were found in the study subjects. Fig. 1A shows representative examples of FERG recordings obtained by SFS in a subject during one experimental session. Individual packets are the result of a smoothing procedure consisting of a three-point adjacent average. The figure also shows the Fourier spectra (the first 8 harmonic components) of individual packets isolated by a discrete Fourier Series. It can be noted that all the individual packets are dominated by the 1F and 2F components. In Fig. 1B, the plots of 1F and 2F amplitude and phase data as a function of the number of recording packet (right) are shown. It can be seen that both 1F and 2F amplitudes show ample fluctuations during SFS. The corresponding phases seem to track, at least in part, these fluctuations.

When looking at the FERG data averaged across subjects, the fluctuations shown above appeared somewhat reduced in their amplitude by the grand averaging, most likely because their phase varied across subjects. The mean amplitudes and phases (±SE, $n = 14$) of the ERG 1F and 2F components plotted as a function of the packet number are reported in Fig. 2. It can be noted that, after the beginning of SFS, 2F amplitude increases modestly in a fluctuating manner, starting from the 1st packet (about 8 s), to reach a maximum approximately at the 15th packet (120 s). Whether the decrease observed at the packet 16th and the relative flat part of the packets 17th to 20th (136–160 s) is part of the fluctuation or an actual decrease is not clear. The 1F component, while showing also some kind of fluctuating behavior does not display a progres-

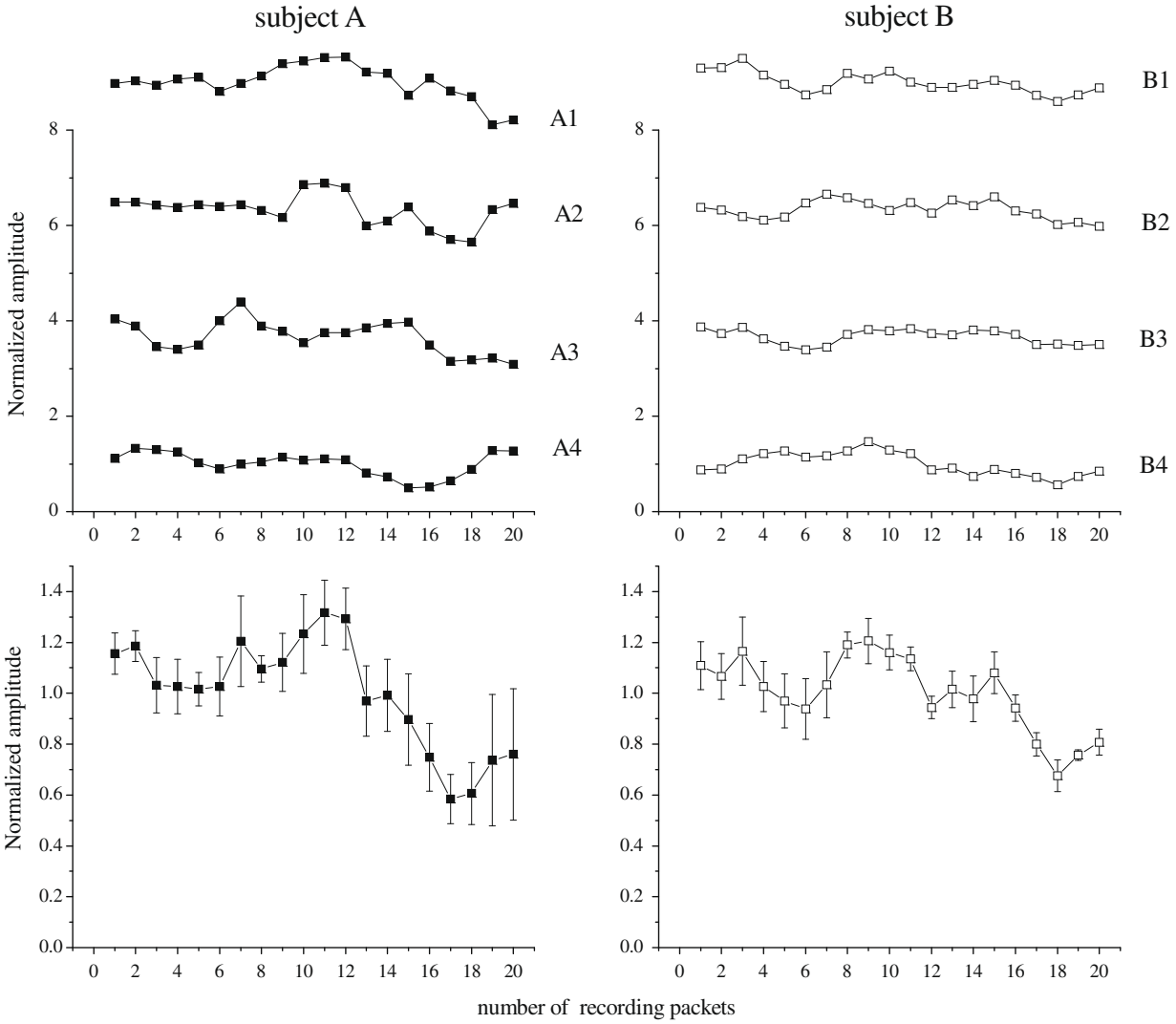

Fig. 3. Subjects A (left) and B (right) performed four times the standard protocol to assess short term reproducibility of the 2F component amplitude of the FERG. The graphs on top are the individual traces (A1 to A4, B1 to B4). Below are the mean values (±SE). The response amplitudes are normalized to the mean value of each recording session (see also Section 2) to allow a closer comparison of individual traces.

sive increase. Clearly, no habituation effect can be observed for either 1F or 2F (see later comparison with PERG). Repeated measures ANOVA showed that mean 2F changed significantly (*F*-ratio: 1.65, df 13,252, $p = 0.041$) as a function of packet number, indicating a short-term effect of SFS on this component. Mean 1F amplitude changes as a function of packet number did not reach statistical significance (*F*-ratio: 1.19, df 13,25, p = ns). Both 1F and 2F mean phase values showed little or no change as a function of packet number.

Fig. 3 shows the results of two sessions where two subjects were studied to assess the within-subjects consistency of SFS response, expressed as the relative (normalized) amplitude variation of 2F component over time. The response amplitudes were divided by the mean value of each recording session (see also Section 2) to allow a closer comparison of individual traces. In all traces, following the initial increase, 2F amplitude demonstrated limited, out of phase amplitude fluctuations. In all sessions an amplitude decrease occurred after about 12–14 blocks giving an overall range of variation of about 30%. Overall, the time course of amplitude fluctuations was poorly reproducible in both subjects.

Fig. 4A shows representative examples of PERG recordings obtained by SPS in a study subject during one experimental session. Individual packets are the result of a smoothing procedure consisting of a three-point adjacent average. The figure also shows the Fourier spectra (the first 8 harmonic components) of individual packets isolated by a discrete Fourier Series. It can be noted that most of the individual packets are dominated by the 2P component. Fig. 4B shows the plots of 2P component amplitude and phase data as a function of the number of recording packet (right). It can be seen that PERG amplitudes tended to decrease, with some fluctuations, during SPS, to reach a value that was approximately half of that recorded at the beginning of the stimulation. Phase appeared to be relatively stable. The behavior of PERG amplitude during SPS appeared rather different from that of the FERG components, either 1F or 2F. This difference can be further appreciated in Fig. 5, where the averaged results of continuous ERG recordings obtained in response to SFS and SPS from six subjects are compared. It can be seen that, while FERG 2F showed only amplitude fluctuations with an increase from baseline of about 30%, the PERG, in agreement with the results reported by Porciatti et al. (2005), displayed a maximum amplitude at the beginning of SPS and then a progressive attenuation until a plateau amplitude was reached. PERG responses can be modeled with an exponential decay function with an average time constant of 2.7 blocks [or ~20 s, see also Porciatti et al. (2005), for comparison].

4. Discussion

The results of Figs. 1 and 2 demonstrate that, during SFS with an 8 Hz sine-wave stimulus presented to the macular region, the light-adapted FERG undergoes only modest changes consisting mainly of an overall amplitude increase of its 2F component during approximately 2 min, followed thereafter by a decrease of this amplitude to values below the initial level. Figs. 1–3 show that the increase was not monotonic, but had a wave-like behavior. The 1F component also showed a non-stationary behavior, although the amplitude did not show an overall increase. No clear habituation effect was found for either FERG components, unlike the PERG which showed the expected habituation effect (see Figs. 4 and 5).

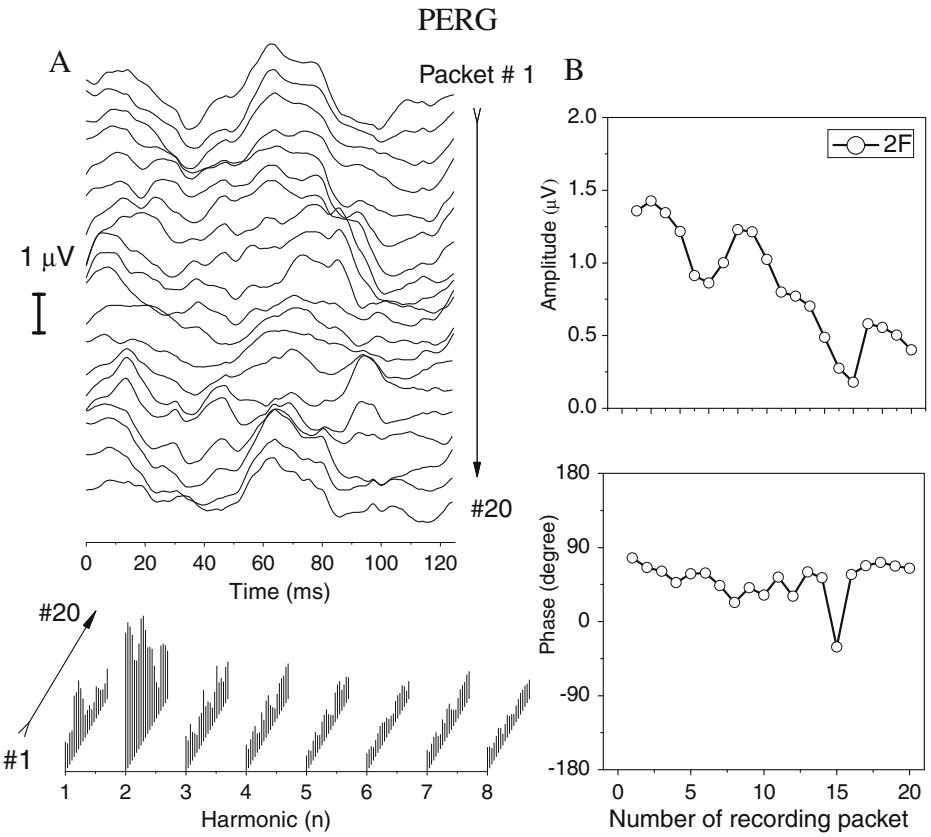

Fig. 4. (A) Representative examples of PERG recordings obtained by SPS in a subject during one experimental session. (Top) Individual packets as resulting from a smoothing procedure consisting of a three-point adjacent average. (Bottom) Fourier spectra (the first 8 harmonic components) of individual packets isolated by a discrete Fourier Series. (B) Plots of 2P amplitude and phase data as a function of the number of recording packet.

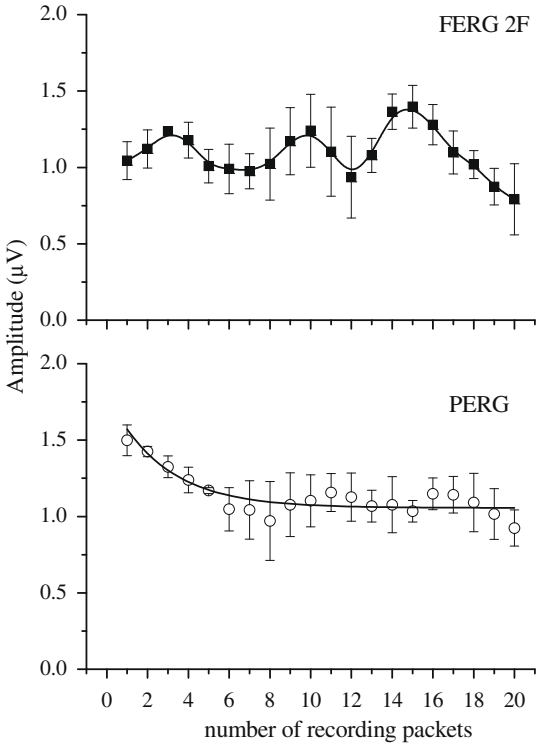

Fig. 5. Results of continuous ERG recordings obtained in response to sustained flicker and pattern stimulation. Average (±SE) of the FERG and PERG 2nd harmonic amplitudes from six subjects plotted as a function of the recording packets. A packet represents 8 s of recording for both FERG and PERG. The PERG response was modeled by an exponential decay function with a time constant of 2.7 recording packets (approximately 22 s).

The fluctuation of the FERG 2F amplitude component during SFS (Figs. 1–3) contrasts with the progressive response attenuation, reported by Porciatti et al. (2005) and also observed in the current study, for the PERG during SPS. Clearly, during the first 2 min of recording, the 2F component does not demonstrate the slow "habituation" effect found for the 2nd harmonic of the PERG during the same period of time. The difference in behavior between the FERG 2F and the PERG may be due to the fact that, although both are dominated by the activity of inner retinal generators (Porciatti and Falsini 1993; Porciatti et al., 1989), these generators may differ. Indeed, selective ganglion cell dysfunction (i.e. descending optic atrophies) affects the PERG but not the FERG 2F (Porciatti et al., 1989). Alternatively, the same generators may differ in their behavior, depending on the stimulus characteristics. It cannot be excluded that if one employed a lower modulation depth, or different temporal frequencies, habituation of the FERG 2F would have also been observed. The present experimental design cannot exclude such possibilities. Finally, given that probably multiple generators contribute to the 2F component (Baker et al, 1988), and they could have a subtractive interaction within the ERG mass response (Porciatti and Falsini, 1993), a possible explanation of the modest increase in 2F amplitude might be the habituation of only one of the 2F sources, revealing an enhancement of the "non-habituating" generators.

The effect observed in this study cannot be ascribed to a progressive amplitude increase as observed for the cone-mediated ERG during light adaptation, since our subjects had already been adapted for 20 min at the stimulus illuminance before starting the experimental recordings. Under this condition of adaptation, the growth in amplitude of the cone ERG is known to be complete (Peachey et al., 1992).

Another possible mechanism that could be invoked to explain the present findings is the adaptive gain control mechanism which has been described for the human flicker ERG (Wu et al., 1995). This process, however, has been characterized by a much shorter time scale (at least 1 s) compared to the effect observed in this study, and to involve both response amplitude and phase. In the current study, mean phase appeared to be rather stable during SFS (Fig. 1).

According to the model of "dynamic equilibrium" used by Porciatti et al. (2005), the lack of the FERG habituation observed in this study may reflect a better ability of the system to maintain a relatively stable energy budget during a physiological stress such as SFS. This is unlike what can be observed for the PERG, but could be similarly related to the vascular hyperemic response induced by SFS in the inner retina (Riva et al., 2005). Interestingly, under specific experimental conditions (Riva et al., 2001; Falsini et al., 2002) the changes in the FERG harmonic components are similar and significantly correlated to the stimulus-evoked changes in optic nerve head blood flow (Riva et al., 2001; Falsini et al., 2002), suggesting a coupling between neural and vascular activity arising from the pooled activity of the post-photoreceptoral cone pathways. The stimulus frequency of 8 Hz was chosen in the present study since this stimulus condition elicits a quasi maximal blood flow response (Riva et al., 2001, 2005), and reveals a significant correlation between this response and the increase in neural activity (Riva et al., 2001; Falsini et al., 2002). A frequency of 8 Hz also allowed a close comparison with the behavior of the PERG during sustained stimulation (Baker and Hess, 1984). Indeed, at around this frequency, the FERG, like the PERG, is dominated by the 2F component, while the 1F has a less favorable signal-to-noise ratio (Porciatti and Falsini 1993; Porciatti et al., 1989; Baker and Hess, 1984) and greater variability (Porciatti and Falsini 1993; Porciatti et al., 1989). Given the retinal origin of the FERG (Baker et al., 1988), the coupling of 1F and 2F with optic nerve head blood flow changes provides support to the hypothesis that the latter parallels the corresponding changes in the neural function of the inner retina. Although these correlations may not directly prove an influence of retinal neural activity on optic nerve head blood flow, it is reasonable to suggest that the pooled response of neural generators, underlying the 1F and 2F components, may induce a vaso-active mechanism resulting in corresponding blood flow changes (Riva et al., 2004, 2005). Relevant to the observed differences between FERG and PERG in the habituation phenomenon is the fact that, in the overall FERG response, a significant contribution comes from generators of the 1F component, unlike the PERG response, where such contribution is present locally but is not detected in the emerging mass signal. If the hyperemic responses to both flicker and pattern stimuli are related to similar large-scale interactions in neuronal activity, a different physiological characteristic may arise in the FERG compared to the PERG.

The flicker-induced neurovascular changes described above may have the goal to provide the energy supply for the electrical/metabolic response of neural generators of the FERG (Falsini et al., 2002; Riva et al., 2005), leading to a condition of "dynamic equilibrium" (Porciatti et al., 2005) where the FERG response is preserved and undergoes only modest adaptive changes linked to corresponding vascular responses. An increase in the energy supply associated with sustained, flicker-induced vasodilation (Riva et al., 2005) may result in an autoregulatory change in retinal activity, which adapts itself to the available energy budget. It may be suggested that the vascular hyperemic response is able to provide the nutritional supply to the FERG neural generators needed to keep constant, or even slightly enhance their electrical activity. Clearly, further studies investigating simultaneously both retinal activity and blood flow are needed to confirm this suggestion. It is worth noting that no habituation was found for the flick-

er-induced hyperemic response in normal subjects (Riva et al., 2005).

It would be of interest to test, in future studies, whether the fluctuations of 2F component shown in Fig. 1 are modified by diseases in which an altered neurovascular coupling has been postulated, such as diabetic retinopathy (Garhöfer et al., 2004) or early glaucoma (Riva et al., 2001). In this case a FERG habituation could take place as a consequence of poor autoregulation. In both diseases a significant loss of the FERG 2F amplitude has been reported (Greco et al., 1994; Falsini et al., 1991) indicating a functional impairment of retinal generators different, at least in part, from retinal ganglion cells.

In conclusion, the present results indicate that the light-adapted FERG does not show habituation under SFS. This may suggest that autoregulatory changes that are linked to the flicker-induced hyperemic response may keep relatively constant the neural retinal activity induced by flicker. The present data may have implications for better understanding the process of neurovascular coupling and its abnormalities in diseases involving retinal circulation and/or metabolism, such as diabetic retinopathy and glaucoma.

Acknowledgements

This study was supported by an unrestricted grant of Merck-Sharp & Dome (to Balestrazzi E.), a grant from Fondazione Cassa di Risparmio in Bologna, Italy (to the Department of Ophthalmology, Prof. E.C. Campos), and a grant from Ministero della Ricerca, Fondi di Ateneo ex 60% (to Falsini B.).

References

Baker Jr CL, Hess RF. Linear and nonlinear components of human electroretinogram. J Neurophysiol 1984;51(5):952–67.

Baker Jr CL, Hess RR, Olsen BT, Zrenner E. Current source density analysis of linear and non-linear components of the primate electroretinogram. J Physiol 1988;407:155–76.

Fadda A, Falsini B. Precision LED-based stimulator for focal electroretinography. Med Biol Eng Comput 1997;35(4):441–4.

Falsini B, Colotto A, Porciatti V, Buzzonetti L, Coppè A, De Luca LA. Macular flicker- and pattern-ERGs are differently affected in ocular hypertension and glaucoma. Clin Vis Sci 1991;6:423–9.

Falsini B, Porciatti V, Fadda A, Merendino E, Iarossi G, Cermola S. The first and second harmonics of the macular flicker electroretinogram: differential effects of retinal diseases. Doc Ophthalmol 1995;90:157–67.

Falsini B, Riva CE, Logean E. Flicker-evoked changes in human optic nerve blood flow: relationship with retinal neural activity. Invest Ophthalmol Vis Sci 2002;43:2721–6.

Garhöfer G, Cawinka C, Resch H, Kothy P, Schmetterer L, Dorner GT. Reduced response of retinal vessel diameters to flicker stimulation in patients with diabetes. Br J Ophthalmol 2004;88(7):887–91.

Ghirlanda G, Di Leo MA, Falsini B, Porciatti V, Marietti G, Greco AV. Detection of inner retina dysfunction by steady-state focal electroretinogram pattern and flicker in early IDDM. Diabetes 1991;40:1122–7.

Greco AV, Di Leo MA, Caputo S, Falsini B, Porciatti V, Marietti G, et al. Early selected neuro-retinal disorder in pre-pubertal tipe 1 (insulin-dependent) diabetic children without microvascular abnormalities. Acta Diabetol 1994;31(2):98–102.

Kondo M, Sieving PA. Primate photopic sine-wave flicker ERG: vector modeling analysis of component origins using glutamate analogs. Invest Ophthalmol Vis Sci 2001;42(1):305–12.

Kondo M, Sieving PA. Post-photoreceptoral activity dominates primate photopic 32-Hz ERG for sine-, square-, and pulsed stimuli. Invest Ophthalmol Vis Sci 2002;43(7):2500–7.

Maffei L, Fiorentini A. Electroretinographic responses to alternating gratings before and after section of the optic nerve. Science 1981;211(4485):953–5.

Peachey NS, Alexander KR, Derlacki DJ, Fishman GA. Light adaptation, rods, and the human cone flicker ERG. Vis Neurosci 1992;8(2):145–50.

Porciatti V, Falsini B, Fadda A, Bolzani R. Steady-state analysis of the focal ERG to pattern and flicker: relationship between ERG components and retinal pathology. Clin Vis Sci 1989;4:323–32.

Porciatti V, Falsini B. Inner retina contribution to the flicker electroretinogram: a comparison with the pattern electroretinogram. Clin Vis Sci 1993;8:435–47.

Porciatti V, Sorokac N, Buchser W. Habituation of retinal ganglion cell activity in response to steady-state pattern visual stimuli in normal subjects. Invest Ophthalmol Vis Sci 2005;46(4):1296–302.

Riva CE, Falsini B, Logean E. Flicker-evoked responses of human optic nerve head blood flow: luminance versus chromatic modulation. Invest Ophthalmol Vis Sci 2001;42:756–62.

Riva CE, Logean E, Falsini B. Temporal dynamics and magnitude of the blood flow response at the optic disk in normal subjects during functional retinal flicker-stimulation. Neurosci Lett 2004;356:75–8.

Riva CE, Logean E, Falsini B. Visually evoked hemodynamical response and assessment of neurovascular coupling in the optic nerve and retina. Prog Retin Eye Res 2005;24:183–215.

Salgarello T, Falsini B, Stifano G, Montrone L, Iarossi G, Balestrazzi E, et al. Morpho-functional follow-up of the optic nerve in treated ocular hypertension: disc morphometry and steady-state pattern electroretinogram. Curr Eye Res 2008;33(8):709–21.

Victor JD, Mast J. A new statistic for steady-state evoked potentials. Electroencephalogr Clin Neurophysiol 1991;78:378–88.

Wu S, Burns SA, Elsner AE. Effects of flicker adaptation and temporal gain control on the flicker ERG. Vis Res 1995;35(21):2943–53.