OP-POWER IN DIABETIC RETINOPATHY

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OP-POWER IN DIABETIC RETINOPATHY

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CONTENTS

PREFACE

The study to be presented here was conducted in the Merwede Hospital in Dordrecht. This hospital has a regional function and accommodates normal and advanced diagnostic facilities. Some of them are so specialized that patients from the whole region come to the hospital for examination.

Why were the conditions at this particular hospital favourable for this study? In a large ophthalmological center, like the Eye Hospital in Rotterdam, the patient population often differs from that encountered in a general hospital. In the former, patients with early signs of diabetic retinopathy will be few and far between. Therefore, an ophthalmological department in a general hospital is a more suitable location for studying the early features of diabetic retinopathy. Since 1981 the author has worked as an ophthalmologist at the Merwede Hospital where he is provided with electrodiagnostic facilities. It was also 1981 that Bert Groeneweg was appointed as a physicist at the same hospital. He developed the software for an interdisciplinary electrodiagnostic department, along with the neurologist Pjotr Carbaat. A central computer system was installed for the averaging, storage and processing of information. Furthermore, the laboratory assistants attached to the neurophysiological department have become familiarized with methods for measuring small physiological signals applying rather complicated equipment. These assistants are also able to perform the visual electrodiagnostic tests. The consultant specialized in internal medicine, Bas van Ouwerkerk, cooperated in the study from the very start. His special interest has been endocrinology and diabetics which has proved to be a favourable condition for the selection of patients according to the protocol. The classical elektroretinographical examination methods were installed and technically completed between 1981 and 1984. On completion plans were made for a research project in collaboration with Professor van Lith of the Erasmus University Rotterdam, based at the electrodiagnostic department of the Eye Hospital in Rotterdam. In this medical institution Professor Henkes was one of the first to apply visual electrophysiology for clinical purposes. Among others he carried out extensive investigations on the electrophysiology of vascular disturbances of the retina. This original work led to the basic interest for

clinical application of electrodiagnostics in the diabetic retinopathy patient.

The basis for my interest in visual electrophysiology was founded during my training period being a resident in the Eye Hospital. The close cooperation of the ophthalmological, internal and neurological departments together with the presence of advanced electrophysiological research facilities served to create the conditions which made the present study possible.

CHAPTER 1

GENERAL CONSIDERATIONS AND A REVIEW OF THE LITERATURE

1.1 Main features of diabetic retinopathy

Patients with diabetic mellitus may develop diabetic retinopathy during the course of the disease. In order to study the electrophysiological characteristics of diabetic retinopathy, it is important to recognise the natural course of the disease in all its various manifestations and to classify the main features into the non-proliferative and proliferative types, as the prognosis will differ according to these two types (Davis, 1974). These features are summarized in table 1 (Murphy and Patz in Little et al., 1983).

Table 1-1. Features of diabetic retinopathy

Non-proliferative (background) retinopathy
Simple background retinopathy
Microaneurysms
Dot and blot haemorrhages
Hard exudates
(Macular edema)
Preproliferative retinopathy
Beaded veins
Soft exudates
IRMAs (see 1.2.2)
(Extensive intraretinal haemorrhages)
Proliferative diabetic retinopathy
Neovascularisation at the disc
Neovascularisation elsewhere in the retina
Fibrovascular proliferation
Vitreous haemorrhage

1.2 Natural course of diabetic retinopathy

The main structural changes in the pathological process of diabetic retinopathy occur at the level of the retinal capillaries, small arterioles and venules. From the evidence currently available, it appears that diabetic retinopathy is initiated by a breakdown of the inner blood retinal barrier, which could be histologically located at the opening of the endothelial tight junctions (Ishibashi et al., 1980). The breakdown of the blood retinal barrier can be visualized by fluorophotometry (Cunha-Vaz, 1981). Another main feature is capillary closure, seen clinically as tiny areas of non-perfusion by means of fluorescein angiography. This method contributes greatly to the diagnosis of microangiopathy. The capillary closures (drop outs) are often recognized as one of the earliest abnormalities in diabetic retinopathy (Klemen et al., 1980). Capillary closure is associated with a dilatation of adjacent patent capillaries, which leads to capillary microaneurysms (de Venecia et al., 1976; Bresnick et al., 1976). An even earlier feature is vasodilatation which is followed by capillary closure. Both features constitute one of the earliest ophthalmoscopical changes of the fundus in diabetic retinopathy (Kohner, 1975). The retinal capillary microaneurysm has become the hallmark of diabetic retinopathy, since its significance was rediscovered in 1943 by Ballantyne and Loewenstein (Duke-Elder and Dobree, 1967).

1.2.1 In 1970, Dobree labelled the tiny changes such as microaneurysms, small haemorrhages and exudates as simple (background) retinopathies. They are often first seen in the posterior pole of the eyes and are generally symmetrically present. The tiny features seen in simple diabetic retinopathy may continue over a prolonged period, sometimes even extending several years (Davis, 1974). In some cases the retinopathy seems to improve spontaneously if judged by its ophthalmoscopical appearance (Oosterhuis et al., 1979; Lauritsen, 1982). The explanation is to be found by studying the evolutionary stages of the microaneurysms. The feature of the microaneurysm begins with a tiny fluorescent spot in the immature stage which developes into a round regular spot, the mature microaneurysm. This stage is followed by an involuting process, showing an irregular fluorescent spot. In the end an atrophic microaneurysm is developed which does not stain with fluorescein (Bresnick et al., 1983).

1.2.2 It is accepted that diminished tissue oxygenation together with abnormal autoregulation may induce the early changes in the retinal vasculature and microcirculation (Grunwald et al., 1984). Another early

sign of microcirculatory disturbances was recognised by Schulte in 1987. By means of fluorotachometry he measured a decreased capillary transit time in the early stages of diabetic retinopathy. It is suggested that the vasodilatation and beading of the venules are indicative of a persistent or a progressive form of the disease (Wilson et al., 1988). In accordance with Wolbarsht et al.'s hypothesis (1981) hypoxic tissue leads to dilatation of the venous capillaries and at the same time to an increase in transmural pressure as well as to a loss in the number of endothelial cells. This forms the instigation to increased permeability, haemorrhages and probably neovascularisation (Stefánsson et al., 1983). The exact nature of the mechanism causing neovascularisation is still unknown, but it is assumed to be complex. Sebag and McMeel (1986) suggested that a retinaderived growth factor plays a role in the angiogenesis.

1.2.3 Preproliferative diabetic retinopathy is a more advanced stage of diabetic retinopathy. It consists of cotton wool spots, beading of the veins, enlarged haemorrhages, some clusters of microaneurysms and intraretinal microvascular abnormalities. These microvascular abnormalities were labelled "IRMA" by Davis (1974). The IRMAs are generally considered to be preproliferative features and are still a sign of intraretinal retinopathy. Preproliferative diabetic retinopathy is a florid stage which progresses rapidly, often within a period of six month, to a proliferative retinopathy.

1.2.4 In proliferative retinopathy, neovascularisations are usually first seen on or around the optic disc, sometimes earlier along the major veins on the retinal surface. Thereupon they may enter into the vitreous. In advanced proliferative stages they lead to glial proliferation and penetrated even further into the vitreous, often causing serious vitreous haemorrhages.

Since the aim of the present study is to assess the role of electrophysiology in diagnosing early diabetic retinopathy, these more advanced proliferative forms of diabetic retinopathy will not be considered.

1.3 Prevalence of diabetic retinopathy

From cross-sectional studies of various diabetic populations it appears that the prevalence of diabetic retinopathy varies according to differences

in race, geographic location, observational techniques and duration of the disease (Constable et al., 1984; Danielsen et al., 1982; Jerneld and Algvere, 1984; Klein et al., 1984a and Kahn and Bradley, 1975). Unfortunately enough, no study representing the Dutch situation is as yet available. Assuming in this respect that the English situation does not differ greatly from the situation in the Netherlands, the English data presented by Kritzinger et al. (1984) give a good impression of what we may expect to occur in the Netherlands. In England the prevalence of diabetic retinopathy in the diabetic population runs at approximately 30%. This estimate has been confirmed in the Netherlands by a small-scale study in a local area of the Netherlands, carried out by Verhoeven (1989). He found a prevalence of 35% diabetic retinopathy among the non-insulin receiving group; of these retinopathies 40% was not found in the general practitioner's records. In a recent report Polak and Casparie (1989) give an estimate for the prevalence of diabetic retinopathy in the Netherlands based on various parallel studies, comprising 60 to 80 thousand patients. In the studies concerning early diabetic retinopathy stereoscopic fundus photographs provide more detailed information than ophthalmoscopic examinations; resulting in a higher prevalence in photographic studies (Moss et al., 1985).

Jerneld and Algvere (1986) performed a photographically controlled study concerning insulin treated patients. They found a prevalence of 56% retinopathy for patients on the Island of Gotland with the onset of diabetes under the age 40, 18% of which consisted of proliferative retinopathy. For the group with onset of diabetes over the age of 40 the prevalence of all kinds of retinopathy together was 40%, including 8.1% proliferative retinopathy. Generally speaking, the longer the diabetic disease lasts the greater the prevalence of all kinds of retinopathy. Kahn and Bradley (1975) as well as Constable et al. (1984) emphasize in their studies the overwhelming importance of the effect of duration and age on the prevalence of diabetic retinopathy. They suggest to use multiple regression analysis to control this effect when studying other factors in relation to diabetic retinopathy. The effect of duration was demonstrated clearly in the Wisconsin epidemiological study by Klein et al. (1984b). For the age group with onset of diabetes over the age of 30 the authors found in the case of the insulin-receiving patients, a prevalence of 38% retinopathy after the diabetes had lasted five years, gradually increasing to 89% after a period of 25 years. For the non-insulin receiving patients' group the prevalence was 25% after 5 years and 58% after 25 years respectively. In a parallel study the age group with onset of diabetes

under the age of 30 showed a prevalence of 25% after five years gradually increasing to 97% after 25 years (Klein et al., 1984c). Only the insulin taking patients, approximately 85% of this group, were examined. Based on incidence data, Klein et al. (1989) calculated sample size requirements. These estimates are highly useful in clinical trials when aiming at investigating the effect of (new) medicaments on diabetic retinopathy.

1.4 Metabolic influences

The influence of a careful metabolic control of the hyperglycemia on diabetic retinopathy has been described in numerous studies. A review of these studies was published by Caird in 1974. He emphasises the positive effect of good metabolic control. The well-known long term prospective study carried out by Pirart (1978) reports less retinopathy, neuropathy and nephropathy in patients with a good control of their blood sugars in the course of 25 years than in those showing a poor metabolic control.

It might be that only the earlier stages of diabetic retinopathy are truly improved by thoroughly kept normoglycemia (Frank, 1984). Reversily intensified insuline treatment may in some cases cause progress of the non-proliferative diabetic retinopathy (Lauritzen et al., 1983; Dahl-Jørgensen et al., 1985). A generally accepted though not proven hypothesis says that in the very early stages of retinopathy the Continuous Subcutaneous Insulin Treatment (CSIT) will decrease the progression of the retinopathy. After treatment with CSIT Hooymans (1986) observed a slight improvement in five out of 35 patients with a simple diabetic retinopathy. The National Health Institute (USA) has designed a major prospective study. This study is meant to provide answers to the questions of how and in what stage a strict metabolic control of the diabetes will influence the progression or amelioration of pre-existent retinopathy (DCCT study group, 1987).

There are several risk factors influencing diabetic retinopathy – high blood pressure being the best known – which aggravate the disease (Klein et al., 1984b and c). In 1985 Shorb reported three cases with severe iron deficiency anaemia. These patients had a rapid progression from a mild diabetic retinopathy to a severe proliferative phase.

The influence of pregnancy on diabetic retinopathy was discussed by

Ohrt in 1984. This study deals with the reversibility of retinopathic changes in the postpartum period.

Other aspects, such as an increased intake of unsaturated fatty acids, have a favourable influence on the natural course of the diabetic retinopathy (Houtsmuller et al., 1980 ; Howard-Williams et al., 1985). The role of alcohol as another risk factor in the genesis of the diabetic angiopathy is a subject that requires further investigation (Young et al., 1984).

1.5 Electrophysiology of the eye

The electrophysiology of the visual system deals with the measurement of electrical signals in relation to various light stimuli on the eye. Three major techniques can be distinguished, viz. the visual evoked potentials (VEP), the electro-oculogram (EOG) and the electroretinogram (ERG).

1.5.1 The VEPs are the electrical cortical responses to visual stimuli. Flashes of light or chequerboard patterns are used. These responses can be led off by electrodes positioned in the occipital region of the scalp. Repeated stimuli have to be used in order to detect them within the background of the electro-encephalogram (EEG). The VEPs are not a subject of the present study.

1.5.2 The EOG measures the potential difference between the anterior and posterior pole of the eye. This dipole consists of a positive polarity at the cornea compared to a negative polarity at the posterior pole of the eye. In ophthalmology the increase of this standing potential to light adaptation is mainly used. It reflects the activity of the deep rather than that of the superficial retinal layers. The EOG is of less significance for the aim of this study, as diabetic retinopathy is predominantly a disease of the superficial retinal layers.

1.5.3 The ERG represents the electrical reaction of the retina elicited by visual stimuli. This electrical response is led off by means of an active electrode at the cornea, to which a contactlens electrode is usually applied with a reference electrode at the earlobe. The ERG consists of various components, of which the three basic ones were originally designated PI, PII, and PIII by Granit in 1933. The ERG basically consists of a biphasic response, a negative a-wave from the receptors (PIII), followed by a positive b-wave (PII) originating in the bipolar cell layer. The late

c-wave (PI) is not always seen. It is a repolarisation potential of the pigment epithelium and is hardly ever applied for clinical purposes. Later, other components were discovered, the early receptor potential (ERP) by Brown and Murakami (1964) and the four small wavelets at the ascending limb of the b-wave named Oscillatory Potentials (OPs) by Cobb and Morton (1953). The OPs are disturbed in early stages of diabetic retinopathy, consequently they are the major subject of this study (figure 1-1).

In the case of the ERG responses their waveforms are expressed by the peak amplitudes in microvolts and by the peak latencies in milliseconds. The peak latency is also called implicit time.

The origin of the separate components has been the subject of many experimental animal studies. They have recently been summarized by Dowling (1987). The a-wave represents photoreceptor activity (Brown, 1968; Miller and Dowling, 1970). The b- wave is located in the Müller cell which extends cross-sectional over a substantial part of the retina (Dowling, 1987). The origin of the OPs are not yet fully understood and will be discussed separately.

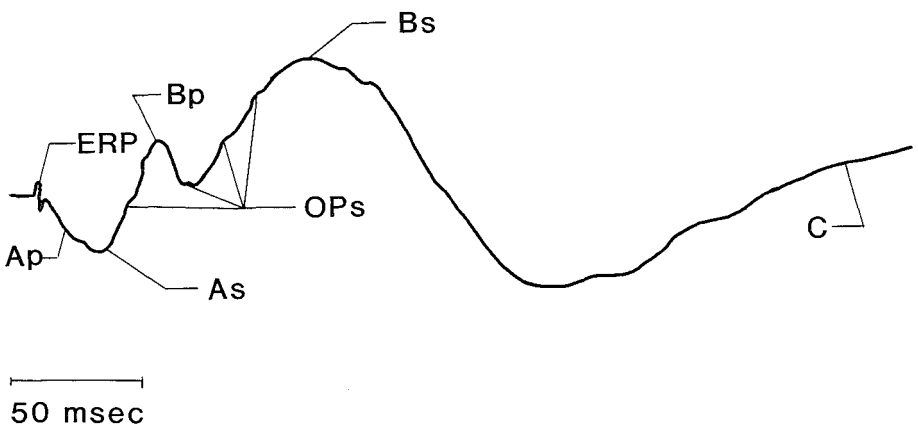

Fig. 1-1: Scheme of ERG components. This drawing depicts the time relations among some of the best-known components in the human ERG. The following components can be seen: the early receptor potential (ERP), the photopic and scotopic a-waves (Ap and As), the photopic and scotopic b-waves (Bp and Bs), the oscillatory potentials (OPs) and the c-wave.

The classical ERG as obtained by luminance stimuli of large retinal areas can be separated into a rod-dominated and a cone- dominated response by applying light stimuli of various luminances and colours . The rods have a maximum sensitivity for blue light near 510 nm and cones for yellow-green light near 555 nm (Krill, 1972). Apart from luminance stimuli, usually elicited with a Ganzfield stimulator, a patterned stimulus can be applied. For clinical purposes, usually a checkerboard stimulus is applied in a reverse way or as a pattern presentation stimulus. The so called Pattern ERG (PERG) is strongly cone driven (Hess and Baker, 1984) and is possibly generated by the retinal ganglion cells (Fiorentini et al., 1981).

1.6 Electrophysiology and diabetic retinopathy

A short historical review will be given concerning the clinical applicability of the ERG in diabetic retinopathy.

The studies of Karpe et al. (1958) and Jacobson (1960) report that diabetic retinopathy does not affect the a- and b-wave amplitudes of the ERG until the disease has reached an advanced stage. In the same study Karpe mentions supranormal ERG b-waves in some diabetic retinopathy patients. The supranormal ERGs were similar to those seen in venous stasis retinopathy. Probably, however, the "supranormal" amplitude is caused by an increase in a-wave amplitude (Speros and Price, 1981). In the late stage of diabetic retinopathy, particularly in the advanced proliferative forms, the b-wave implicit time increases and later on the amplitude decreases. This is followed by alteration of the a-wave (Li et al., 1985).
In 1962, Yonemura et al. proved the clinical significance of the OPs elicited by a strong single flash. It appears that the diminution of the OPs occurs prior to the stage in which retinal destruction is extensive enough to diminish the b-wave amplitude. The extent of the Müller cell (1.5.3) and its dominant role in the b-wave generation might explain why the b-wave is not as sensitive as the OP to retinal capillary ischaemia is (figure 1-2) (Dowling, 1987). In these early clinical studies the correlation between the seriousness of the retinopathy and the reduction of the amplitude of the OPs remained uncertain.

In 1966, Simonsen found the OPs to be hypernormal in diabetic patients without visible retinopathy. In the same study he found reduced OPs in eyes with simple retinopathy. The studies of Algvere (1968) and

Gjötterberg (1974) confirmed the reduction of the OP amplitudes. Furthermore, they established that in later stages and especially in the proliferative stage of diabetic retinopathy the a- and b-wave are also diminished. Galloway et al. (1972) paid special attention to the early stage of diabetic retinopathy and found normal OPs in a considerable proportion of such cases. More data concerning the relationship between OP amplitude and the severity of the retinopathy were presented by Bresnick and Palta (1987a), Hennekes and Deschner (1984) and Wanger and Persson (1985). None of these investigators could establish OP amplitude reduction in diabetic eyes without any visible retinopathy.

In 1981, Simonsen suggested that the OPs have predictive value. In this study 54% of the patients belonging to the subnormal group of OP activity developed a proliferative retinopathy within six to eight years, while from patients with normal OP activity this was only the case in 6% over the same period. More recently, this was confirmed by Bresnick and coworkers (1984, 1987b). They estimated a ten-times higher rate of progression within one to three years in the diabetic retinopathy group with reduced OP activity, when compared to the retinopathy group with normal OPs. Unfortunately, they did not give the duration of the diabetes for the separate groups, although we know from prevalence studies that duration and progression rates are important factors (Klein et al., 1989).

1.6.2 Not only the OP amplitudes but also the temporal aspects may provide information in relation to diabetic retinopathy.

Brunette and Lafond (1983) found, under photopic conditions, an increase in the implicit time of the OPs before the OP amplitudes were reduced in the early stages of retinopathy. Bresnick and Palta (1987c) made similar observations and found the implicit times of the second and third OP to be increased in established stages of retinopathy. They also compared the implicit time of the b-wave to a 30 Hz flicker with the OP amplitude reduction and found the latter less sensitive than the increase in implicit time of the b-wave in the established and advanced stages of retinopathy.

1.6.3 The perimetric function has already presented a decrease of retinal sensitivity in patients with a non-proliferative diabetic retinopathy as was demonstrated with an octopus perimeter by Bell and Feldon (1984). This decrease in sensitivity often corresponds with capillary dysfunction as controled by fluorescein angiography. This relation, however, could not

always be established. Probably there are also other metabolic influences on the retinal function which can not be related to capillary non-perfusion alone (Sannita et al., 1988).

1.6.4 As to the PERG, Arden et al. (1986) found an amplitude reduction in patients with a preproliferative diabetic retinopathy. He describes the amplitude reduction of the PERG to be more sensitive compared to the OP amplitude reduction. This observation, however, could not be confirmed by Coupland (1987) and Wanger and Persson (1985). They demonstrated the reverse, that the PERG is less sensitive than the OPs in background retinopathies. This means that the PERG reveals only additional information in the preproliferative stage of diabetic retinopathy. Probably the diabetic maculopathy is a precondition for abnormal PERGs, since mainly the central field contributes to the PERG (Schuurmans and Berninger, 1985). In the present study the PERG is not tested, since this method is not assumed to be as sensitive as the OP measurement in the early stages of diabetic retinopathy and secondly for the practical reason that the test procedure became too extensive for the diabetic patients participating the study.

1.7 Origin of the Oscillatory Potentials

Most observations performed to discover the origin of the various components of the ERG are carried out on animals. Yonemura and Hatta (1966) studied the OPs in the frog and Yokoyama et al. (1964) in the rabbit.

The OPs are of low amplitude in these animals. They were supposed to originate from structures in the inner nuclear layer. In primates Brown (1968) obtained relatively better developed OPs at the periferal retina in comparison with the much smaller foveal OP amplitudes. By experimental clamping of the retinal circulation and separate depth profile studies he located the OPs in the inner part of the retina. In monkeys, Ogden (1973) could separate the origin of the OPs from the b-wave and found their maximum activity at the level of the inner plexiform layer.

The experiments on the clamping of the retinal circulation are indicative of the sensitivity of the OPs to disturbances of the retinal circulation (Hayreh, 1984). The case report on an arterial occlusion points in the

same direction (van der Torren et al., 1988). Steinberg stated in his Friedenwald lecture (1987) that the mitochondria of the rods are especially sensitive to mild hypoxia. This may be due to the anatomical configuration, namely, the close packing of the inner segments and mitochondrial clustering in the rod inner segments. In these experiments the sensitivity to the choroidal circulation and retinal circulation were not separated. Another explanation may be that the circuit from the bipolar cell to the ganglion cell is extremely sensitive to hypoxia and to small disturbances in the glycolysis (Winkler, 1981; Steinberg, 1987).

A major question in studying the OPs is whether the separate wavelets originate in the same structure and whether they will react similarly in pathological conditions. These questions are especially important in electrodiagnostics, since they determine, whether or not the OPs may be added up and expressed in one measure.

Wachtmeister and Dowling (1978) supposed different neuronal origins for the separate wavelets which together are called the OPs. The first wavelet was believed to originate more proximately within the retina than the following OPs. Later on, however, Heynen and van Norren (1985) found similar current source density profiles of all OP components in monkeys. These authors demonstrated that the bipolar cells most likely elicit the OPs. Wachtmeister subscribed to their conclusion in a study carried out with these investigators (Heynen, Wachtmeister and van Norren, 1985). Based on these latter studies, it has been assumed for the present study that the OPs can be averaged and expressed on an equivalent basis in terms of power. Without undermining this assumption, it is also possible that other intraretinal structures may influence the OPs. Another possibility for their origin might be the interplexiform cell (IPC), which belongs to the amacrine cell system (figure 1-2). These IPCs are supposed to form a feedback circuit of the horizontal cell onto the bipolar cell terminals and to contain dopamine as a transmitter (Dowling, 1987).

After Heynen's investigations (1985), pointing to the bipolar cell as the only origin of the OPs, Miyake et al. in 1988 posited a relation between the OPs of the parafoveal ERG and amacrine distribution. The arguments in favour of the influence of the amacrine cells on the generation of the OPs was also brought forward by Wachtmeister and Hahn in 1987. Since it is known that the retina contains dopaminergic neurons (Ehinger, 1982) and that the generation of the OPs can be influenced by a dopamine antagonist (Wachtmeister 1981a and b), the IPCs may also be responsible

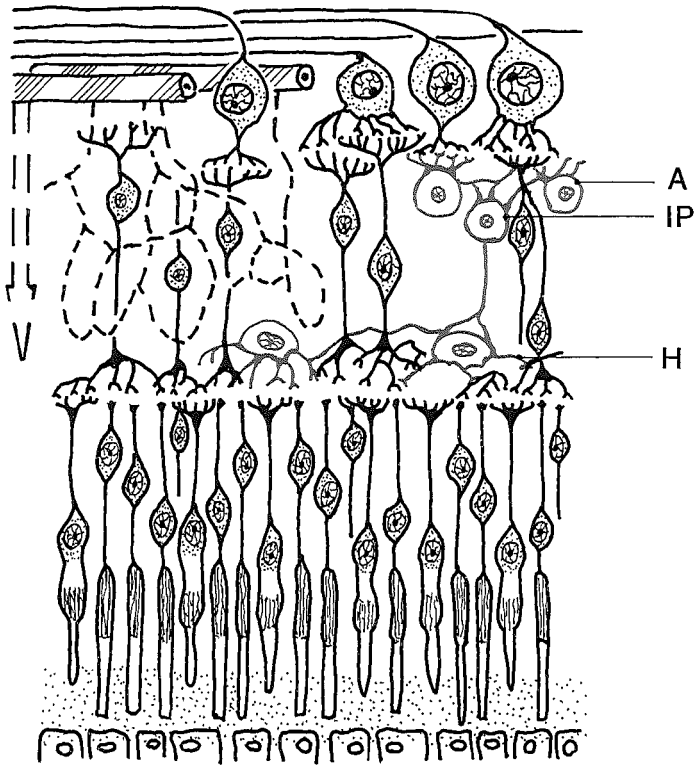

Fig. 1-2: The cell types of the amacrine system (green), as viewed in a vertical section of the retina. Three types are drawn in: A: the amacrine cell, H: the horizontal cell, IP: the interplexiform cell.

for this effect (Dowling, 1987). The effect of metoclopramide (dopamine) on the Ganzfeld ERG was studied by Jaffe et al. (1987). He concluded that dopamine could even modulate the OP biogenesis in human retina. The neurotransmitter L-acetylcarnitine is another drug with cholinergic properties which can influence the OP peak-latencies in diabetic patients (Sannita et al., 1988).

1.8 Electroretinogram in photocoagulated eyes

Xenon and Argon photocoagulation has become an accepted treatment of proliferative diabetic retinopathy (DRSR group, 1978). The purpose of this therapy is to destroy a substantial proportion of the peripheral retina in order to diminish oxygen demand and consequently the stimulus to neovascularisation (Korner and Korner, 1988). Several investigators tried to evaluate the effect upon the retina produced by extensive photocoagulation by means of ERG recordings. Ogden et al. (1976), Liang et al. (1983) and Perlman et al. (1985) found considerable reductions in the a- and b-wave after panretinal photocoagulation. The reduction in these studies was comparable to the extent of the destruction of the retinal area. Gjötterberg and Blomdahl (1981) and Li et al. (1986) saw a better correlation when the photocoagulated area was located in the periphery than when it was located more centrally in the retina.

In a study concerning the alteration of the OPs in diabetic retinopathy after photocoagulation of large retinal areas, Hennekes and Deschner (1984) demonstrated a slight increase of the OP amplitude among some of a group of 28 subjects. The authors took this to be a positive result arising from the treatment. Furthermore, they suggest that OP measurement provides prognostic value in relation to functional recovery after photocoagulation treatment. Their results are based on the measurement of the second OP, which is a difficult technique, since the second wavelet is often hardly recognizable in the advanced stages of diabetic retinopathy (chapter 4).

1.9 Aim of the present study

It is generally accepted that the ERG reflects retinal activity as a mass response and is widely used as an objective index of retinal function. As such it can also be applied to measure the retinal function in diabetic

retinopathy. From the previous review of the literature it is obvious that the conventional ERG can only detect diminished retinal function when visible retinopathy is present; these ERGs have no prognostic value.

Whether the OPs may provide such information in the early stage of retinopathy can not definitely be answered as yet. In the preproliferative stage this correlation has been demonstrated by Simonson (1981) and Bresnick and Palta (1987).

Until recently, the OPs were assessed by simple recognition of the separate wavelets without measuring them. Because of the difficulty in recognising the individual OP wavelets, the present study intends to develope an index that facilitates the distinction of the OPs from the a- and b-wave and provides a quantitative measure.

The study is devided into three sections:
1: Searching for an improvement in the quantification of the OPs.
2: Applying the measurement technique in a representative population of diabetic patients.
3: Comparing the new technique with methods applied up to now.

CHAPTER 2

MEASUREMENT OF OSCILLATORY POTENTIALS

A pilot study

Partly published in:

K. van der Torren,G. Groeneweg, G. van Lith (1988)
Measuring oscillatory potentials: Fourier analysis
Documenta Ophthalmologica 69: 153-159

This chapter describes a new OP-recording technique by means of signal processing. The method was tested on a reference group and compared with the results obtained from patients with diabetic retinopathy.

2.1 Introduction

Various techniques have been applied to detect and to assess the Oscillatory Potentials (OPs) of the electroretinogram (ERG). The early study carried out by Cobb and Morton (1953) revealed that the human ERG evoked by strong single light flashes produced several rhythmic wavelets superimposed on the ascending limb of the b-wave. In 1962, Yonemura et al. validated their clinical interest by merely observing these single flash responses. He found the OPs to be lowered or absent in diabetic retinopathy.

In order to obtain a quantitative measurement Algvere (1968) used a short time constant isolating the wavelets from the b-wave. Later on, Stodtmeister (1973) attempted to improve this technique by introducing an analog filter.
The disadvantage with these filtering techniques is the resulting time shift in the OPs. Furthermore, there is an existence of a substantial residual activity of the a- and b-wave. Miyake and Solish (1978) tried to calculate the individual amplitudes, applying a calculated midpoint technique with a computerized system. The sum of the amplitudes was the measure for the OP activity.

Also temporal aspects had the interest of various investigators (Kojima and Zrenner, 1978; Bresnick and Palta, 1987c; Lachapelle, 1988). Recognition of the separate waves is obligatory if peak latencies are to be measured. Often this appeared to be difficult in a clinical setting as pathological conditions and artifacts reduce the possibility of recognizing the wavelets separately. Special attention has been paid to Algvere and Westbeck's study (1972), in which Fourier analysis has been applied. For clinical practise this method was further developed in this study. Furthermore, Wachtmeister's recommendations (1973) concerning the luminance of the stimulus and a stimulus interval of 30 sec were used from the outset. Later on the stimulus interval was increased to favour the scotopic component as described in chapter 3.1.2.

2.2 Apparatus and method

2.2.1 Hardware
A PDP 11/23 computer (Digital Equipment) was used to store the ERG recordings. With this computer system a filtering procedure could be

selected and the filtered data could be compared with the unfiltered original recordings.

2.2.2 Signal processing

The high frequency OP signal was separated from the low frequency a- and b-waves by a Finite Impulse Response filter (FIR) of 110- 250 Hz (Rabiner and Gold, 1975). This filter has the advantage of a linear phase characteristic and does not cause a phase shift in the OPs. From this filtered signal the implicit times as well as the amplitudes of the four OP wavelets could be measured separately (figure 2-1). These separate OP wavelets are labelled OP1, OP2, OP3 and OP4. The peak latencies (implicit times) are determined from the start of the light flash to the positive peak of the separate OP wavelets, using an automatic top detection. The amplitudes of the OP wavelets are measured from the minimum of the preceding negative trough to the maximum of the following positive wavelet.

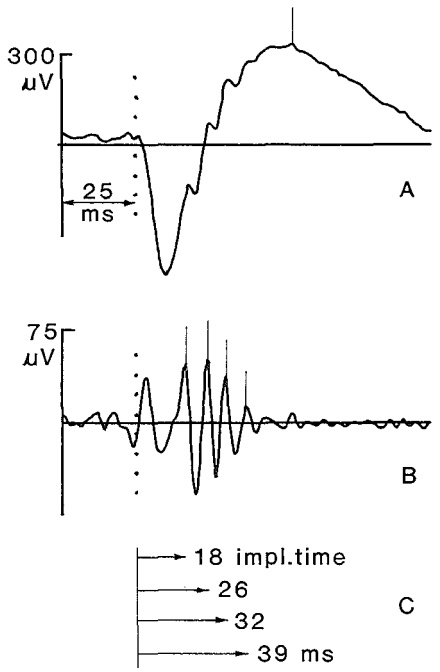

Fig. 2-1: Normal single flash ERG; white light flash of 10 J.
Horizontal: time base. Vertical: microvolts.
A) unfiltered signal, B) signal after finite impulse response filtering procedure (a = a-wave component), C) the implicit times of four OPs.

In the FIR filtered signal some residue from the a-wave is present, though there is a difference in frequency. Figure 2-1B presents this a-wave component (a) preceding the OPs. The frequency of this component may approach 90 Hz, which can be near the lower frequencies of the OPs. The OPs are ranging from 123-170 Hz (chapter 2.4.2). Fourier analysis is applied to separate the OPs from this a-wave activity, yielding a frequency spectrum from which the OP-power is calculated. In figure 2-2 the power densities at various frequencies are shown in the third column (FFT). A maximum is to be seen near 155 Hz. At this maximum the dominant frequency can be located. The OP-power is calculated from the power density spectrum around this dominant frequency using a floating window. The frequency range within this window is limited by the frequency at which the energy has half the intensity of the energy at the dominant frequency (figure 2-2, shaded area).

2.2.3 Electroretinography

The classical light flash ERG is measured under scotopic and photopic conditions using a Ganzfield stimulator (van Lith et al., 1973) and a Henkes contactlens electrode. The ERG signal is 2000 times enlarged by an amplifier developed by van Heuningen, Goovaerts and de Vries (1984). The amplified signal passes an analog filter between 0.5 and 500 Hz after which the signal is sampled with a 12 bits AD (analog/digital) converter (ADF01 type DEC) at a frequency of 2000 Hz. The digitized ERG signal is stored and processed using a separate procedure.

The pupils have always been dilated beforehand. After 15 minutes of adaptation to the dark the scotopic ERG is elicited by means of blue light flashes of 1 Joule, the luminance of which is lowered by neutral density filters up to 2 log units. This procedure is followed by the recording of the OPs with single white light flashes of increasing energy, viz. 1, 10 and 80 J respectively. The 10 J flash stimulus is repeated three times to

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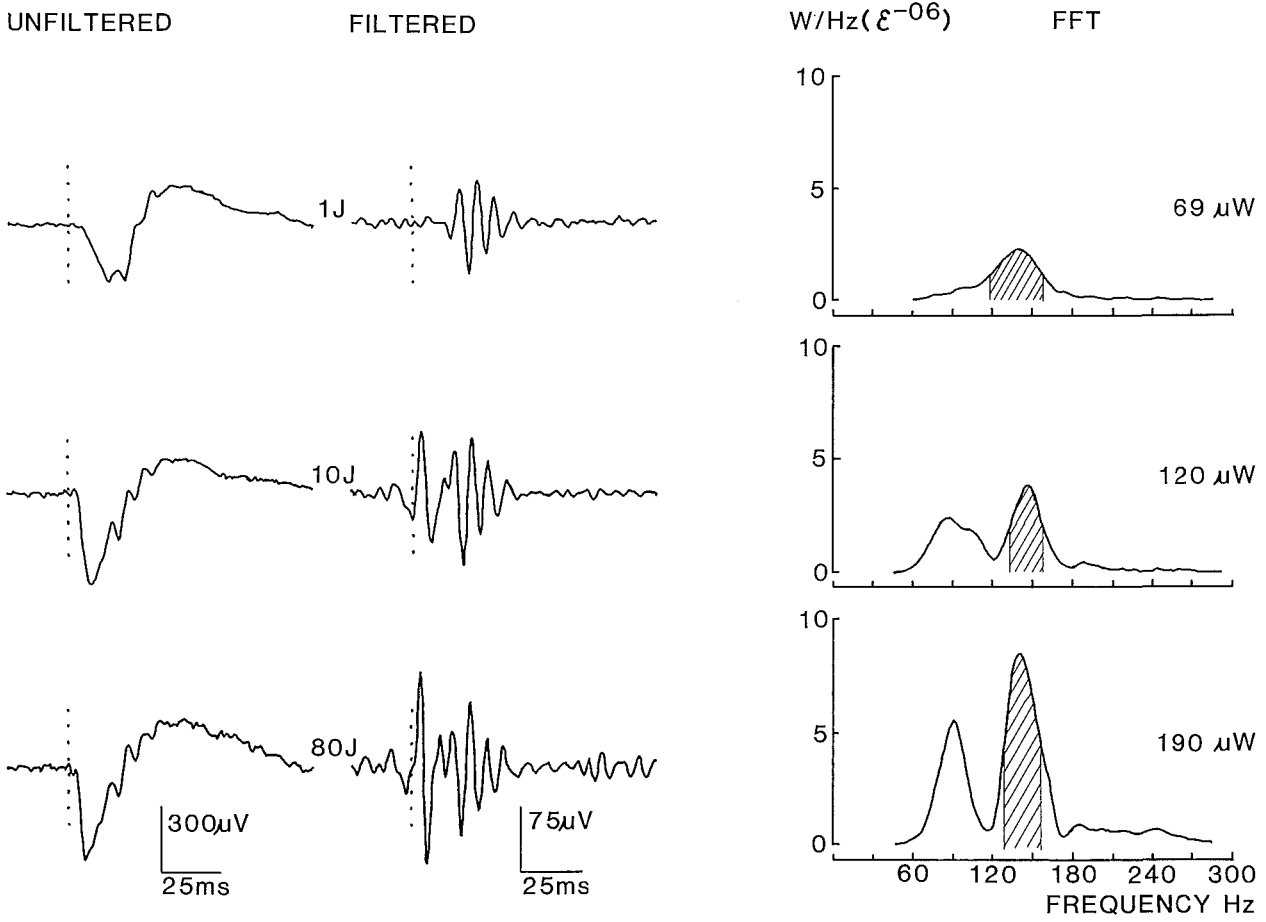
Fig. 2-2: Single white flash ERGs of a normal subject with intensities 1, 10, and 80 J. First column: the unfiltered signal; second column: the finite impulse response- filtered signal; third column: the fast Fourier transform (FFT) signal wherein the shaded interval is used to calculate the OP-power; fourth column: the OP-power in microwatts.


UNFILTERED
FILTERED
W/Hz(ε−06)
FFT
1J
10J
80J
300μV
25ms
75μV
25ms
10
5
0
69 μW
120 μW
190 μW
60
120
180
240
300
FREQUENCY Hz

measure reproducibility. Between the registrations of the OPs a three minute interval is maintained. After the OP measurement the photopic responses are determined applying white light flashes of 1 J with a frequency of 2 Hz, superimposed on a bright blue background of 6.5 cd/m2 .

2.2.4 Subjects
The reference group consisted of 13 healthy subjects with 25 normal eyes matched up with the diabetic group in terms of age. The latter consisted of 12 patients with 23 eyes, varying in age between 30 and 65 (mean age 41) and with diagnosed diabetes for at least seven years. The diabetic retinopathy patients had not been treated with xenon or argon coagulation before examination. By fluorescein angiography five patients in this group appeared to have non-perfusion areas or small neovascularisations.

2.3 Results

2.3.1 Power spectrum presentation
Figure 2 gives the frequency spectrum of a single white flash ERG with OPs obtained from a normal subject with the three light flashes of increasing energy. In the unfiltered signal the increase of the OP amplitudes is evident. This is more clearly presented by the Fast Fourier Transform (FFT), the power of which can be expressed in one measure, the microwatt.

The results of two diabetic patients are compared with those of a normal subject in figure 2-3. The reduced OP activity of the patient with early diabetic retinopathy is already visible in the original signal. In this patient only a few microaneurysmata and haemorrhages were visible by ophthalmoscopy, assessed as a simple diabetic retinopathy (chapter 1.1). Nearly absent OP activity is seen in the original recordings of the other patient with proliferative diabetic retinopathy in its early stage. This patient has small prepapillary proliferations, representing an advanced

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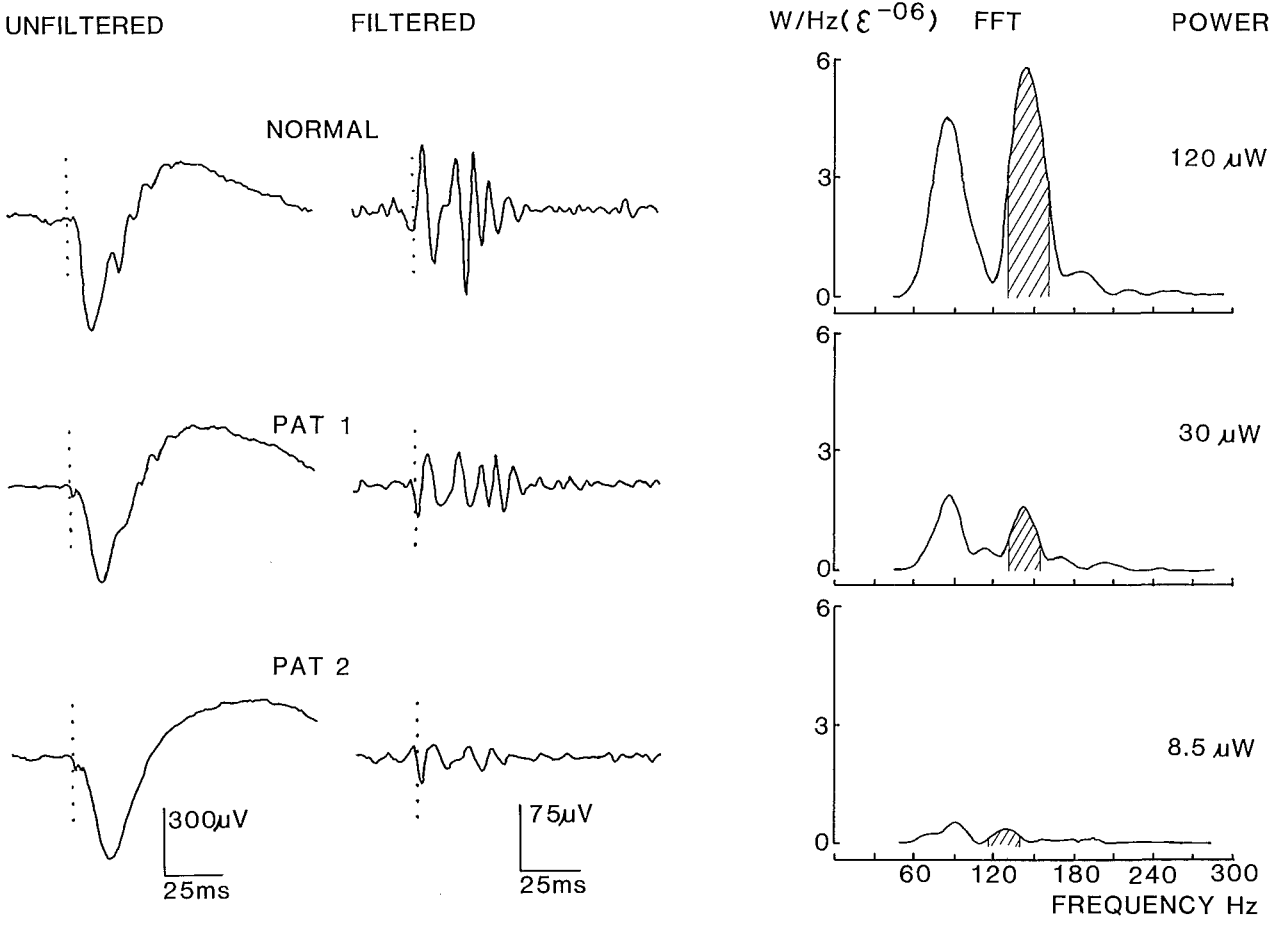
Fig. 2-3: Single white flash ERGs a normal subject (upper curve) and two diabetic patients: patient 1 with early diabetic retinopathy (middle curve), patient 2 with proliferative diabetic retinopathy (lower curve).


UNFILTERED
FILTERED
W/Hz(ε⁻⁰⁶)
FFT
POWER
NORMAL
PAT 1
PAT 2
300μV
25ms
75μV
25ms
120 μW
30 μW
8.5 μW
6
3
0
60
120
180
240
300
FREQUENCY Hz

diabetic retinopathy and belonging to the proliferative form (chapter 1.1). Although the reduced activity can be seen in the original signal, the difference is more pronounced in the FFT; while the power provides the numerical value.

Expressed in terms of power the results of the 10 J flash (figure 2-2) appeared to be most suitable when compared with those of the 1 and 80 J flashes. The OPs of the 1 J flash are not sufficiently pronounced, whereas in this pilot study the 80 J flash appeared to be too much of a burden for the patient.

2.3.2 Power spectrum distribution

The distribution of the power measurement obtained from the 25 normal eyes and the 23 diabetic eyes are presented in the bar diagram of figure 2-4. For the horizontal axis a logarithmic scale has been chosen, since the values of the diabetic patients are relativily small compared to those of the reference group. From this figure one can deduce that the diabetic data are diminished. Most of them are well separated from the reference group. Ten of the twenty-three diabetic eyes had non-perfusion areas the

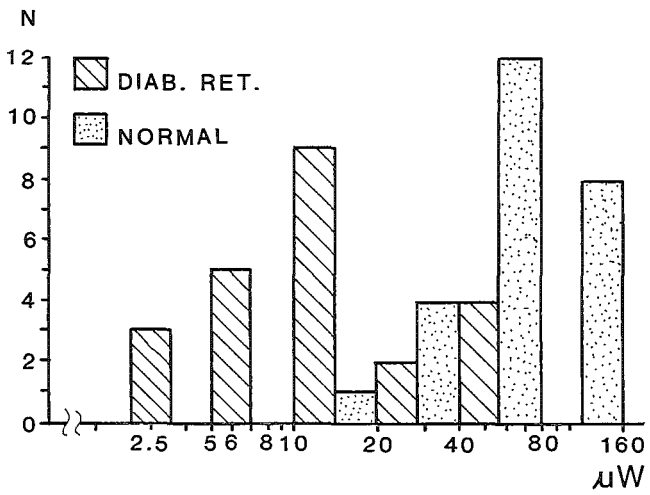


Fig. 2-4: Survey of results: normal subjects (dotted bars) and patients with diabetic retinopathy (shaded bars). Horizontal line, power in microwatts; vertical line, number of patients.

other ones varied from an early to a proliferative stage. There was at least a ten times difference between the power of the established diabetic retinopathy subjects and the power of the normal subjects, promising a power range wide enough to separate the various pathological stages of retinopathy. This was not performed in the pilot study but will be described in chapters 3 and 4.

2.4 Discussion

2.4.1 Computerized system

The advantages of a computerized system have been briefly discussed by Miyake et al. (1978). The ERG storage and automatic top detection makes signal processing practicable. Kozak (1982) made us aware of the possibility of entering a second computation procedure after the digital filtering of the original signal. In the present study the computer system was a great asset in selecting the optimal filtering procedure as well as in selecting the frequency interval for the power measurement.

2.4.2 Selection of the frequency interval

By isolating the OPs on a frequency basis, a measurement was introduced which takes into account not only the maximum OP energy at the dominant frequency, but also the distribution over the frequency domain. This is different from a selection on the basis of time only, which forms the basic principle for most of the clinically applied methods. By introducing the frequency in the OP measurement method more information on the characteristics of the OPs is obtained.

Theoretically a fixed frequency interval for the OP-power calculation would be preferable, as the contribution from the foregoing a-wave to the power in a fixed interval is supposed to be more constant than in a floating interval. Therefore, we first opted for the procedure of calculating the power with a fixed interval from 123 to 170 Hz. This choice was based on the average width of the area, in which OP activity could be observed in the FFT. To select the limits of lower and higher frequency for this fixed interval, the mean and standard deviation (SD) of the upper and lower frequencies was calculated. The mean was 135 Hz for the lower and 160 Hz for the upper frequency. The lower limit was obtained by reducing the lower frequency by twice the SD of 6 Hz, the upper by adding twice the SD of 5 Hz. In this way an interval of 123 Hz to 170 Hz was obtained for the fixed interval procedure. When applying this interval for calculating the power in the group of normal individuals, it proved impossible to

obtain a Gauss distribution for the reference group. For this reason we opted for a floating window.

A normal Gauss distribution was obtained as long as the calculations were based on the dominant frequency with a floating window centered around this frequency and with a window aperture dependent on the curve width. When applying a floating window the spectrum width is taken into account. In this way it is possible to obtain extra information from the OP-power when the frequency spectrum of the OPs is broadened. This would not be possible if only the dominant frequency was calculated without a window.

The dominant frequency of the diabetic patients will be further analyzed in the next chapter.

CHAPTER 3

POWER MEASUREMENT OF THE OSCILLATORY POTENTIALS IN DIABETIC RETINOPATHY

Partly published in:

Torren van der K, Lith van G (1989).
Oscillatory potentials in early diabetic retinopathy.
Doc. Ophthalmol. 71: 375-379.

In this chapter a description is given of the effect of diabetic retinopathy on OP-power.

3.1 Introduction

3.1.1 OP measurement in diabetic retinopathy

The notion that abnormalities of the OPs could be used as prognostic tools in diabetic retinopathy was put forward by Simonsen (1975). Various functions of the OPs, such as temporal aspects (Kojima and Zrenner, 1978; Bresnick and Palta, 1987c; Lachapelle, 1988) and amplitude summation (Algvere, 1968; Miyake et al., 1978) were studied in relation to the progression of retinopathy. The results of the methods applied in the foregoing studies do not resolve the question as to whether reduction in OP activity is a sign of an early stage of diabetic retinopathy. In the present study a quantitative method based on the frequency spectrum of the OPs is applied (chapter 2). Since dark adaptation has a substantial influence on the OPs, special attention has been paid to this topic (3.1.2). The sensitivity of the method has been tested by examining patients in the early stages of diabetic retinopathy. It is for this reason too that a special section has been devoted to the classification of diabetic retinopathy (3.1.3).

3.1.2 Dark adaptation and OP measurement

In order to obtain high OP responses Wachtmeister (1973 and 1987) advocates to register OP activity at the transition from scotopic to photopic vision. This can be achieved by presenting high energy light flashes (>1 J) with a stimulus interval of 30 seconds (Wachtmeister, 1973 and 1987).

Under scotopic conditions Peachy et al. (1987) observed similar OPs in rod monochromats and normals. Measured at the transition from photopic to scotopic vision, the OPs in the rod monochromats were markedly reduced. This finding suggests that under these conditions the OPs result primarily from the cone system. From the studies carried out by Gliem and Schulze (1966) as well as Frost Larsen et al. (1981) the sensitivity to dark adaptation appeared to be significantly reduced in the case of diabetic retinopathy. This reduction appeared to be progressive towards a proliferative stage. This finding is confirmed by the fluorescein angiographic studies of Niki et al. (1984) as well as Shimizu et al. (1981). In both studies it is reported that examination of the mid-peripheral area is very important for the detection of early diabetic retinopathy. It is also this area where rod density is highest and where dark adaptation is predominantly determined (Sigelman and Ozanics, 1982, in Duane). In 1988 Sieving and Nino studied the Scotopic Threshold Response (STR)

in the ERG and concluded that this response reflects post-receptor processing in the rods pathway. In a recent study Aylward (1989) describes the STR as a sensitive parameter to early diabetic retinopathy.

A conclusion from these studies is that the dysfunction of the rod system is a characteristic of early diabetic retinopathy.

For this reason recording OPs under scotopic conditions seems to be more commendable. It was King Smith et al. (1986) who made a clear distinction between scotopic and photopic OPs. His observations are often taken into consideration.

To enhance the scotopic component a three minutes stimulus interval was applied in this part of the study instead of the 30 second interval advocated by Wachtmeister (1987).

Another advantage of favouring the scotopic component is the frequency shift of the OPs to higher values as reported by Wachtmeister in 1973. This shift makes it easier to discern the OPs from the lower frequencies of a- and b-wave.

3.1.3 Classification of diabetic retinopathy

As the aim of this study is to detect the role of OPs in early diabetic retinopathy, a special classification of the early stages was needed.

The report on the classification of diabetic retinopathy by Haut et al. (1987) demonstrates clearly the advantages of a system based on fluorescein angiography. One of the generally applied systems is the Airlie House Classification (DRS group, 1981). In accordance to this classification, a four field photographic standard was chosen for the present study. However, since the Airlie House Classification is less adequate in discriminating the early stages of diabetic retinopathy, more specified criteria for the early signs have been introduced.

The following studies influenced the choice of these criteria. The photographic classification system as presented by Nielsen (1984) specified the early signs of diabetic retinopathy in detail but did not take into account the additional information, which can be obtained from fluorescein angiography. The early signs, which are not ophthalmoscopically visible, were studied by Klemen et al. (1980) by means of an advanced fluorescein angiographic technique.This investigator found

capillary non-perfusion areas in 29% of the elderly and in 60% of the juvenile diabetic pre-retinopathies. A similar angiographic study relating to non-perfusion areas was presented by Niki et al. (1984), whereby abnormalities in the periphery were observed in only four of the 152 eyes. Therefore, the fact that these peripheral changes might be missed in the four field photographic standard as used in the present study does not pose a problem. The capillary non-perfusion areas are sometimes called dropouts. Actually, dropouts are caused by capillary closures, which are different from the non-perfused areas, caused by arteriolar closures. The latter sometimes showing cotton wool spots (Bresnick et al. in Little et al., 1983). Dropouts or, in other words, non-perfused capillaries did not add to the predictive value of his regression models (Bresnick and Palta, 1987c).

In early diabetic retinopathy the avascular fovea may be enlarged under fluorescein angiography, due to dropouts at the inner ring of the capillaries surrounding the fovea. In these cases, this enlarged avascular area is again surrounded by capillaries with enlarged dilatations

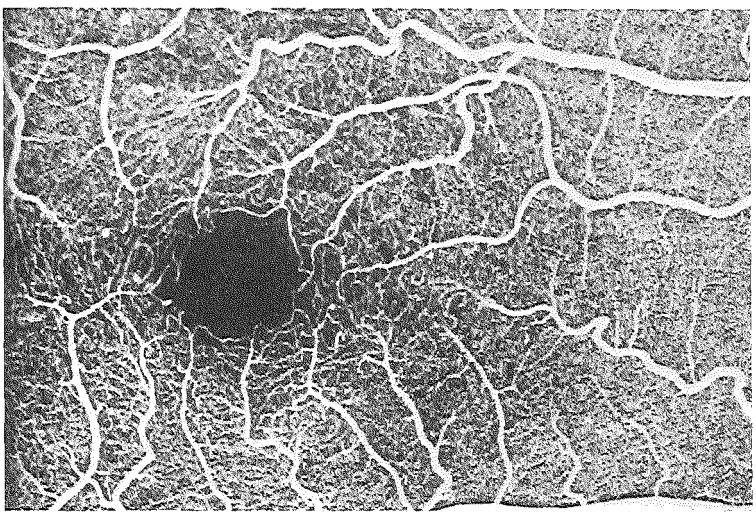


Fig. 3-1: Fluorescein angiogram of the foveal area of the right eye. Increased visibility of some capillaries surrounding an enlarged avascular zone. This picture is called: "too good to be true".

(Bresnick et al. in Little et al, 1983). These two phenomena create the impression of an enlarged and more visible foveal area. These foveal changes are sometimes labelled "too good to be true" (figure 3-1). In the present study they could be observed in the first group of diabetic patients.

3.2 Method

3.2.1 Diabetic classification

The fundus photography was performed with a Topcon TRC-50 VT type camera. Both eyes of all the patients participating were photographed, followed by fluorescein angiography using four standard fields with a visual angle of 35 degrees and one central field with temporal extent of 50 degrees. The four standard fields are : the optic disc area, the regions along the upper and lower temporal vessels, and a central area (figure 3-2). Based on these pictures four groups of patients with diabetic retinopathy could be distinguished.

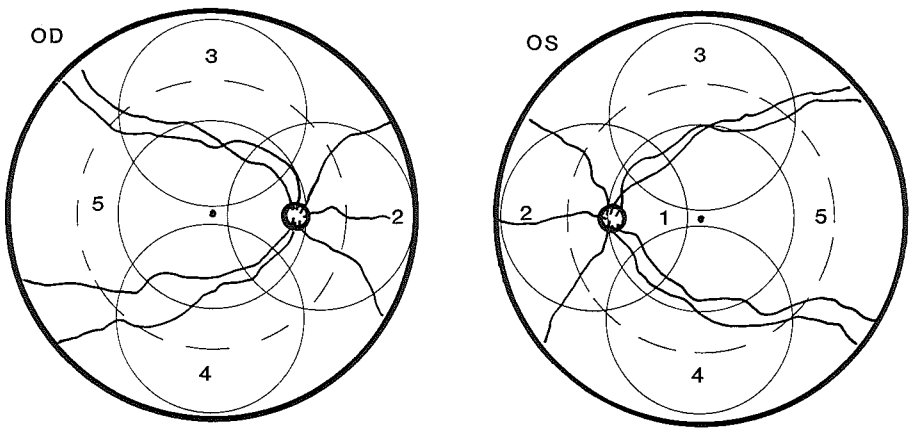


Fig. 3-2: The four fields of fluorescein angiography. The photograph angle is 35 degree for the fields 1 to 4, photographed in the first 50 seconds followed by the 50 degrees central field

Diabetic retinopathy groups 1-4;

Group 1: *No ophthalmoscopical visible diabetic retinopathy;*
Some microaneurysms are permitted, but no more than five for the cumulated area of the four standard photographs.

Group 2: *Early exudative diabetic retinopathy (non proliferative);*
Microaneurysms, some haemorrhages, exudates, capillary non-perfusion areas.

Group 3: *Preproliferative diabetic retinopathy;*
Scattered intraretinal haemorrhages, exudates, cotton wool spots, beaded veins, large dark blot haemorrhages, clusters of microaneurysms surrounding non-perfusion areas, IRMA's.

Group 4: *Proliferative diabetic retinopathy;*
Intraretinal and preretinal proliferations beginning proliferations on the optic disc.

Group NS: *Normal subjects.*

The patients were assigned to the diabetic classification before the electrodiagnostic procedure was performed. The assignment to the four categories was checked by a colleague specialised in fluorescein angiographic diagnostics.

3.2.2 Patients selection

The reference group (NS) consisted of 27 healthy subjects matched for age with the 81 diabetic patients as far as this was possible. The age is slightly different distributed due to a practical problem; hospital staff was particularly easy to motivate when it came to finding normal subjects for the determination of the normal values. However, the mean and range of the parameter age for this group was smaller than that of the diabetic patients. The mean age of the reference group is 41.5 years with a range from 20 to 60, while that of the diabetic patients is 43.2 years ranging from 20 to 65 years. Taking group 1 separately, the mean age is 40. The age distribution of the entire diabetic group (1-4) and that of the reference group NS is given in figure 3-3. Group 1 is also shown separately since the OP-power data between this group and the reference group are also compared separately.

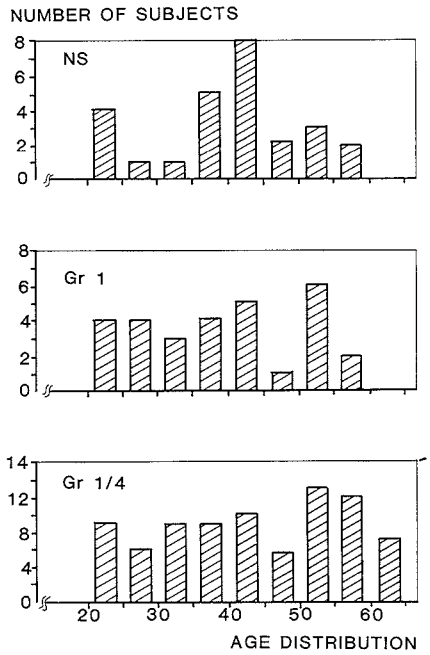


Fig. 3-3: Age distribution divided into classes at five year intervals. Upper recording: normal subjects (NS), middle: diabetic group 1 and lower: total of the four diabetic groups.

In a recent paper by Sannita et al. (1988) it has been shown that OP amplitudes change with age. He found an increasing OP amplitude with a maximum at about 50 years of age, slowly decreasing thereafter. The effect of age on the OP-power of the diabetic groups and the reference group was tested separately by the Wald test which measures the significance of fixed effects and covariates. The age effect appears to be non significant. The 81 diabetic patients (42 male and 39 female) had been treated with insulin (65 out of 81) or with oral antidiabetics (16 out

Table 3-1. Mean duration of diabetes per group of retinopathy

Classification	Mean in years
Group 1	12
Group 2	15
Group 3	15.5
Group 4	18

myopia of seven diopters; in the case of another patient, the ERG is computed from only one eye due to the presence of a cataract on the contralateral eye. None of the patients is known to have other eye diseases. In particular they were not suffering from glaucoma. These features are important, since it has been reported that the development of diabetic retinopathy is inhibited by high myopia and glaucoma (Becker, 1981). Glaucoma may also decrease OP activity (Gur et al., 1987). Cataract will influence the degree of retinal illumination brought about by the stimulus. For these reasons patients with either of these abnormalities were barred from the study, except for the one with the myopia. Also patients with more general afflictions which might show possible side effects, such as established hypertensive or coronary heart disease, were excluded from the study.

3.2.3 Electroretinographical method

The classical light flash ERG, filtering procedure by finite impulse response filter (FIR) and power measurement were performed as described in chapter 2 ← . The scotopic OPs were obtained by a 10 J single white light flash following the standard scotopic ERG with an additional three minute interval of dark adaptation before each flash. The dominant frequency of the FFT was noted separately. The photopic OPs were obtained from the averaged 1 J photopic ERG as described in chapter 2.2.3.

For the detection of the classical ERG in diabetic patients the Henkes contact lens electrode has been recomended by Vey et al. (1979). In the first period of the study this contact lens sometimes produced photo-electrical artefacts (figure 3-4) (Carr and Siegel, 1982). The frequencies of these artefacts were sometimes near or in the same interval as the OP frequencies. Other artefacts were probably caused by a shifting of the contact lens over the eye, due to discomfort and muscular spasm during the large dark-adaptative period. Other disadvantages of long examination procedures and shifting of the contact lens were corneal erosions as seen more often in the diabetic group, probably due to a decrease in the quality of the epithelium attachment to the basement membrane of the cornea (Azar et al., 1988). To avoid these problems during the study the circular inner curve of the contact lens was modified into an eliptic one by the Titmus contact-lens factory.

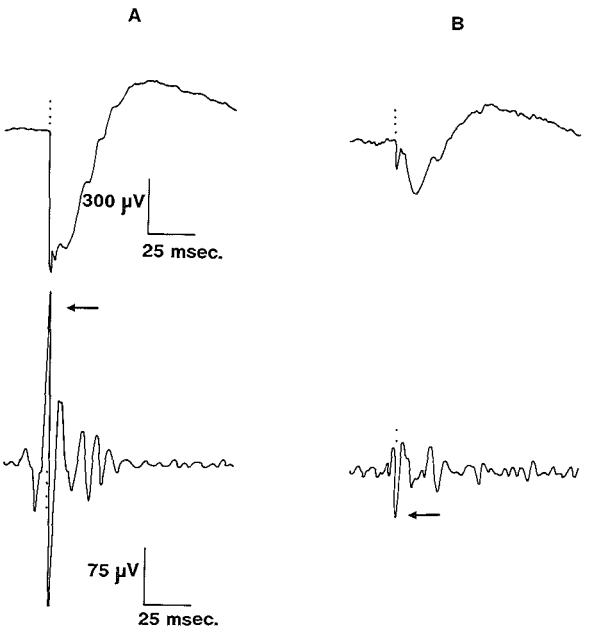


Fig. 3-4: Single flash responses with photoelectrical artefacts in the first milliseconds. The FIR filtered signal shows activity within the frequency area from 110-250 Hz.

3.3 Results of OP-power measurement

3.3.1 OP-power and diabetic retinopathy

The frequency spectrum and calculated power of four patients, one from each of the specific diabetic groups, are given in figure 3-5 to serve as an example. Parallel to the increasing severity of the diabetic retinopathy, OP activity, expressed in OP-power, decreases gradually. The OP-power of the reference group and the four diabetic groups together ranged from 29 to 153 microwatt and from 3 to 149 microwatt respectively. This indicates that the inter-individual OP-power data are spread over a wide range not only in the diabetic groups but also in the reference group.

The mean of the OP-power and the standard deviation (SD) of the groups are given in table 3-2. The SD is large with respect to the mean of the OP-power, which can be explained by the large variation range per individual and a relativily small sample size (N). The OP-power of the diabetics and normal subjects is worked out on a logarithmic scale, because it resulted

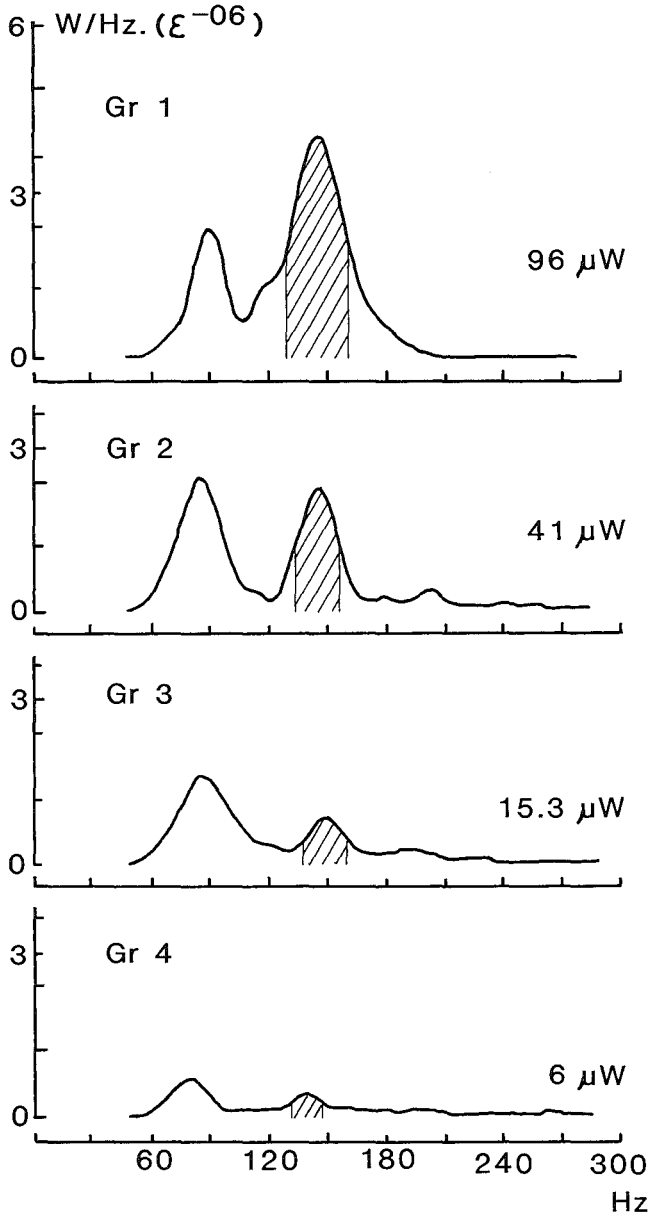


Fig. 3-5: Fast Fourier Transform (FFT) and calculated power of a 10 Joule single white flash ERG in four diabetic patients, one from each group.

Table 3-2. Mean and SD of the OP-power in microwatts seperately for right and left eyes; N: Number of eyes.

	OD			OS		
Classification	Mean	SD	N	Mean	SD	N
NS	58.5	19.2	26	54.3	23.7	26
Group 1	42.4	26.4	28	42.3	26.7	23
Group 2	37.5	29.8	26	41.6	33.5	21
Group 3	17.1	9.9	15	16.9	10.1	11
Group 4	9.7	5.9	6	7.7	3.9	5

in a better distribution in terms of the reference group (see chapter 2.4). This choice was confirmed by the statistical results. The correlation coefficient of the paired samples t-test is slightly higher with a logarithmic presentation (reproducibility, chapter 3.3.3). The measurement results of the OP-power per diabetic group are to be found in the bar diagram numbered figure 3-6. It appears that the OP-power is lower in diabetic group 1 than in the normal group. This means that the OP activity diminishes even before the appearance of diabetic retinopathy. The statistical estimates concerning the four diabetic groups and the reference group were calculated by a repeated measurement ANOVA with covariates (Statistical software program BMDP 5V 1988). The interdependent variable in this procedure is the logarithm of the OP-power measured in microwatts for both eyes. The group factor is the factor between subjects (Beta) from the diabetic groups (1-4) and the reference group (NS). The factor within subjects represents the variation between the left and right eye and the covariate represents the age of the subject. Due to a number of values among the OP-power results which were missing, a maximum likelihood estimation method had to be applied. These results were missing for technical reasons, namely that some subjects had only one eye tested either OD or OS, whilst others had values rejected following the discovery of artefacts. During the research period the chance of obtaining such artefacts was reduced by remodelling the contact lens electrode as described before (3.2.3). Figure 3-3 presents an example of a very high response in the FIR filtered curve, caused by an

artefact. As one can see, this artefact is situated within the frequency range of the filter, being 110-250 Hz, and as a consequence it may also disturb the OP-power measurement.

3.3.2 Statistical estimates between the separate diabetic groups

Table 3-2 gives the mean and standard deviation values of the OP- power for the two eyes seperately and for all groups. The irregularity in the number of patients per group (N-values) are a result of the fact that for some patients only one eye could be examined. The overall relation of the four diabetic groups on the OP-power of both eyes was statistically significant ($p < 0.001$), when tested with the repeated measurement ANOVA. Table 3-3 gives estimates of the factor Beta (the group variable) and of the reference group (indicated as group NS). The Beta coefficient for the group NS is set at zero. The coefficient decreases inversely with the severity of the diabetic retinopathy. Furthermore, the power calculation method reveals a statistically significant decrease of OP activity in diabetic patients without visible retinopathy, the p-value being 0.004 for the difference between group 1 versus the NS group. The p-values for the successive steps of the remaining groups are given in the right- ← hand column of table 3-3. The OP-power estimates of table 3-3, already present a clear reduction in OP activity at a stage in which no ophthalmoscopical changes were to be seen. As already explained, this does not necessarily imply the absence of diabetic retinopathy, since by using fluorescein angiography small changes can sometimes be seen (chapter 3.2.1).

If in the estimates of table 3-4 the reference is set at 100% for the normal group (NS), then the OP-power of the first diabetic retinopathy group is 65%, the second group 54% , the third group 26% and the fourth group 17%. The estimates for the OP-power which were recalculated from the factor Beta are given in the right-hand column of table 3-4. These estimates are adjusted for left and right eye as a within-patient factor and for the age of the subject as a covariate.

Fig. 3-6: Distribution in classes of the OP-power in log microwatts, the reference group and the diabetic group 1 , 2 and 4 (shaded bars oblique slanting to the right), group 3 shaded bars oblique slanting to the left.
NS= normals, I= diabetic group 1, II= diabetic group 2, III= diabetic group 3, IV= diabetic group 4.

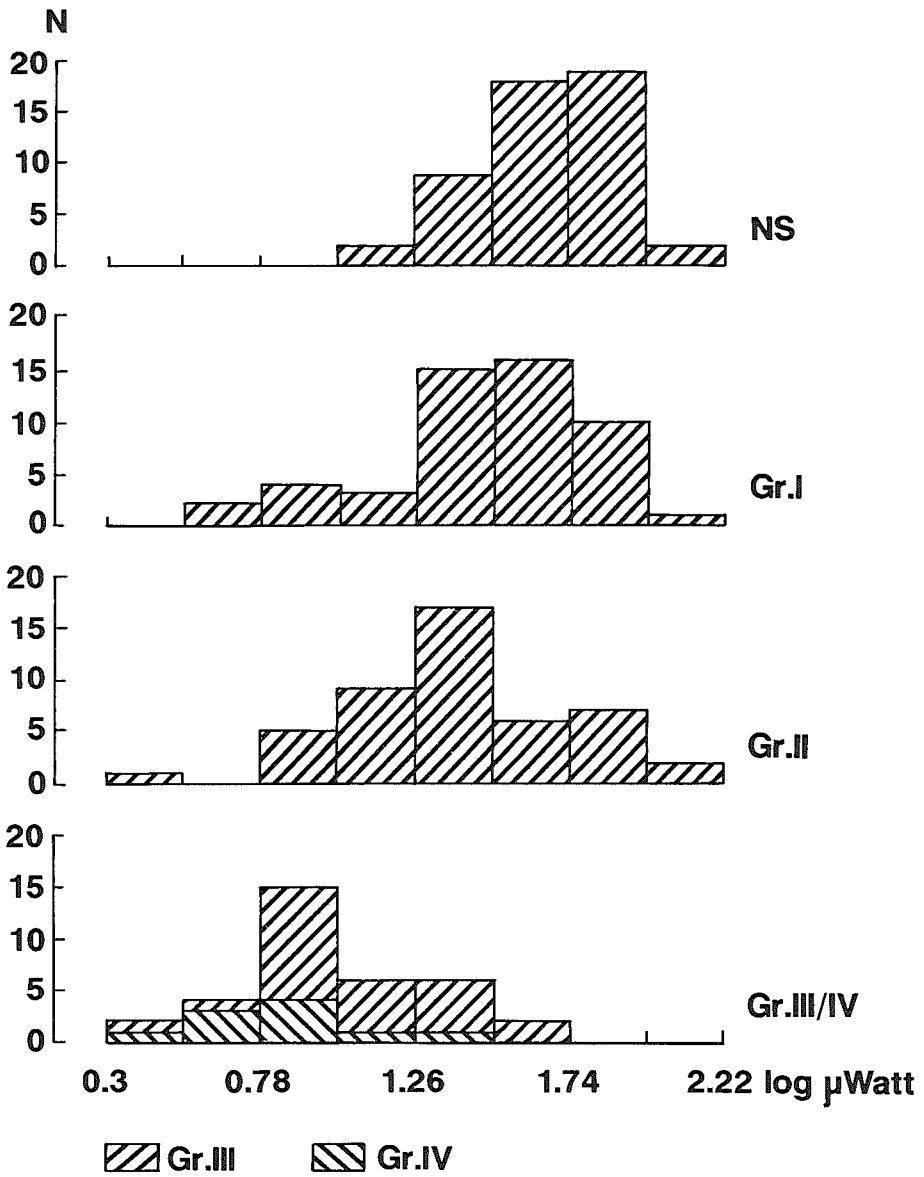

N
20
15
10
5
0
NS
Gr.I
Gr.II
Gr.III/IV
0.3
0.78
1.26
1.74
2.22 log µWatt
Gr.III
Gr.IV

Table 3-3. Estimates of β coefficient from separated measurement ANOVA

Classification	β coefficient	Level of significance
NS	0	
		P = 0.004
Group 1	–0.42776	P < 0.001
		P = 0.126
Group 2	–0.61706	
		P < 0.001
Group 3	–1.33517	
		P = 0.051
Group 4	–1.80226	

Table 3-4. Estimates of OP-power in percentages and in microwatts.

Classification	%	μW
NS	100	54.9
Group 1	65	31.7
Group 2	54	20.2
Group 3	26	15.1

3.3.3 Reproducibility

To test the reproducibility of the OP-power, the 10 J response was recorded three times, of which the first and the second were used for the OP-power calculation, the third response was only taken when an artefact was present in one of the other two recordings. The difference in OP-power of the second response was statistically tested with a paired t-test and with the Wilcoxon matched pairs Signed-ranks test. Neither test indicated a difference on repeated measurement. Also the correlation coefficient (R), from the paired sample t test was calculated between the duplicate measurements, R being 0.887 for the OP-power and 0.920 for the corresponding logarithm values. This also implies that there was no displacement in the order of ranking. The correlation coefficient for the logarithm of the OP-power was slightly higher for both eyes which

designates a preference for this setting (chapter 2.4 and 3.3.1). One may conclude that there is a stable reproducibility for the individual OP-power measures.

3.3.4 Dominant frequency

The dominant frequency of the FFT shifted to lower values, while the diabetic retinopathy increased in severity. Table 3-5 presents the mean and SD of the dominant frequency of the OPs for the right eye as recorded in all groups separately. The left eye showed similar values. The frequency shift to lower values was found to be statistically significant for the successive groups as tested by the Kruskal-Wallis 1-way ANOVA. This implies that the frequency can be considered as a parameter in addition to the implicit time (chapter 4.2.4) and the OP-power.

3.3.5 Photopic OP measurements

When applying the frequency spectrum method to measure the OPs of the photopic ERG, it appeared to be impossible at the time to separate the OPs from the foregoing a-wave by using Fourier analysis, as the frequencies of these two components are too close. The situation under photopic conditions is different from the one under scotopic conditions in that the OPs photopically do not present themselves as distinct peaks although they are clearly visible in the unfiltered and FIR filtered recordings.

In those cases where a distinct interval could be obtained the mean frequency of the photopic OPs was 124 Hz. In one third of the cases in

Table 3-5. Mean and SD of the dominant frequency in Hz from the FFt for the rigt eye; N: number of eyes.

Classification	Mean	SD	N
NS	148.2	5.8	26
Group 1	142.4	5.8	28
Group 2	141.6	5.7	26
Group 3	137.2	6.1	15
Group 4	135.6	6.8	7

which OPs could not be detected by Fourier analysis, the frequency was near or below 100 Hz.

3.4 Discussion

The diagnostic value of the OPs in diabetic retinopathy has been discussed in the past by several authors. Yonemura et al. (1962) made us aware of the clinical applicability of the OPs. They observed that in cases with simple retinopathy the a- and b-wave were not disturbed, whereas the OPs could be greatly diminished or might even disappear. In the present study the OP activity of the diabetic group 2 was already reduced to 54% of the reference group (table 3-4) confirming the observations of Yonemura and Kawasaki (1978), since the patients they examined could be classified as belonging to diabetic group 2 (chapter 3.1.3 and 1.1).

Simonsen (1975) found a reduction in amplitude as a function of duration of the diabetes. In 1980 he reported the prognostic value of the reduced OPs with respect to the risk of developing proliferative diabetic retinopathy. In cases with greatly reduced OPs, he found an increase from simple to proliferative diabetic retinopathy within three years. In the present study a greatly reduced OP activity of 26% of the normal value was estimated for the diabetic group 3 consisting of preproliferative diabetic retinopathy patients (table 3-4). This finding can be seen as a confirmation of the prognostic value since the preproliferative diabetic retinopathy stage is supposed to deteriorate rapidly to a proliferative stage (Davis, 1974). Bresnick and Palta (1987a) reported a positive relation between the reduction in the summed amplitudes of the OPs and fluorescein leakage in the angiograms. No reduction of OPs was found in the diabetics without leakage, in contrast to the observations of the present study. Based on a longitudinal study the authors compared the predictive value of summed OPs to the predictive value of the regression models of the ETDRS study group (Bresnick and Palta, 1987b). They also found a clear relation between the reduction in amplitudes of the OPs and non-proliferative diabetic retinopathy.

In the present study there was a clear relation and overall fit between the OP-power and the retinopathy classification. This fit was statistically highly significant. In 1987 Coupland observed a reduction in the summed amplitudes of patients without diabetic retinopathy. This study was checked with colour photographs but without fluorescein angiographs.

Some small lesions such as microaneurysms and small haemorrhages, visible by ophthalmoscopy, were permitted in the non-background retinopathy group of the Coupland study. In the present study these patients would be classified as belonging to group 2.

In all preceding studies no clear description can be found of a reduction of OP activity in patients without any diabetic retinopathy signs. Fluorescein angiography is often missing. Furthermore, Kothe, Lovasik and Coupland (1989) stated that OP decrease is not easy to prove, as variability of the repeated measurement is large under various stimulus conditions; 25% in his study. Although the OP-power also has a large range, a statistically significant reduction can be established already between the normal subjects and the patients of group 1, who have no ophthalmoscopic sign of diabetic retinopathy.

This seems to indicate that the OP-power measurement is a sensitive method for registering function reduction in diabetic retinopathy, even at a stage at which hardly any anatomic lesion can be observed by fluorescein angiography. The p-value for the difference between groups 1 and 2 is somewhat less than between group 1 versus NS and group 2 versus 3, which may well be due to the slight dissimilarity in retinopathy severity between these two groups. The transition from group 2 to 3 is better defined by the criterion of non-perfusion, which explains the more pronounced statistical separation of the OP-power values between these two stages. Concerning the even more severe stage of group 4, it is well known, that the OPs are nearly absent in the proliferative stage of diabetic retinopathy (Lawill and O'Connor, 1973; Li et al., 1985). The very low estimates for the diabetic group 4, the mean being 6.2 micro-Watt (table 3-3), are consistent with these earlier observations.

The dominant frequency has a tendency to decrease with the progression of diabetic retinopathy. In some cases the OP-power data were minimal as was the case with the patients of group 4, being those with proliferative diabetic retinopathy, although it was possible to identify the dominant frequency for the OP-power measurement. Even a case which had hardly any OP activity left at all could still be included.

It is important to test the reproducibility of the OP-power, since Kothe et al. (1989) found unstable repeated measurements of OPs in several conditions of light adaptation. Probably the reproducibility factor needs to be looked at more carefully in studies where the OP technique is

applied in accordance with the standard ERG protocol of the International Standardisation Committee of the ISCEV (1989), or when using conditioning flashes. These circumstances are not scotopic but probably even photopic (Peachy et al., 1987). Under scotopic conditions the reproducibility was stable as was statistically substantiated by the Wilcoxon test as well as the paired t-test (reproducibility, chapter 3.3.3).

Measurement of OP-power under photopic conditions appeared to be less than feasible. Often there was not a clear distinct frequency interval between the OPs and the foregoing a-wave. The main reason was the decrease of the OP frequency from approximately 145 Hz scotopically to somewhere between 127 and 100 Hz photopically. Another reason may be that the frequency of the a-wave itself increases. The marked decrease of the OP frequency photopically is in accordance with the findings of Wachtmeister (1973), Gur and Zeevi (1980). Application of coloured stimuli to elicit cone dominated OPs may possibly solve these problems (King Smith et al., 1986).

A comparison with conventional methods of OP recording will be given in chapter 4.

CHAPTER 4

A COMPARISON BETWEEN CONVENTIONAL OP REGISTRATIONS AND OP-POWER METHOD

This chapter consists of two sections; the first deals with a comparison between OP-power registration and the conventional technique for visual assessments of OP recordings. The second describes the amplitude and implicit time measurement of the wavelets OP2 and OP3; it was suggested recently that this technique would lead to an improvement in OP recordings (Bresnick and Palta, 1987c; Coupland, 1987a).

4.1 Visual assessment of the OPs compared to OP-power

4.1.1 Conventional OP registration

The assessment of OPs by visual inspection is the conventional method in use for clinical interpretation. By recording the OPs it is possible to differentiate between disorders of the deep receptoral and the superficial postreceptoral retinal layers. In the latter the OPs are lower at an early stage. The most common postreceptoral disturbances are those relating to retinal circulation. Except in the case of diabetic retinopathy, the OPs have usually disappeared or diminished by the time the acute stage of venous and arterial occlusions has set in (van Lith, 1980). In advanced cases of glaucoma, diffuse uveitis and Behçet disease, in which medial opacities can blur funduscopic examination, the detection of OPs can reveal additional information for clinical use (Yonemura and Kawasaki, 1978; Speros and Price, 1981). The postreceptoral disturbances manifested in congenital stationary night blindness with myopia (Völker-Dieben et al., 1974; Lachapelle et al., 1983) and X-linked juvenile retinoschisis (Krill, 1972), are less frequently seen in the ERG laboratory, but are sometimes important for the establishing clinical diagnosis. The visual assessment of OPs is a clinical method which has proven to be adequate enough for the diagnosis of advanced cases.

This method is still in use in the electrodiagnostic laboratory of the Rotterdam Eye Hospital. This gave us the opportunity to compare this conventional method to the newly developed OP-power technique.

4.1.2 Visual assessment of the OPs

The OPs are visually assessed and classified by a colleague, who is specialised in visual electrodiagnostics. As shown in the schedule (table 4-1) four categories can be distinguished. Figure 4-1 shows a single flash ERG curve; one for each category.

Table 4-1. The four categories of visual assessed OPs.

category 1: well developed OPs	- the OPs have a clearly descending limb in each wavelet
category 2: lowered OPs	- the normally descending limb is running more or less horizontally
category 3: just identifiable OPs	- the normally descending limb is changed into an oblique ascending direction
category 4: absent OPs	- the b-wave is smooth without possible identification of OPs

For this assessment and the OP-power calculation the same recordings have been applied.

4.1.3 Comparison of visually assessed OPs with the OP-power

Figure 4-2 gives the mean and range of the OP-power for the four categories. As one can derive from the figure, there is a large variation in the OP-power especially in the categories 1 and 2. The Pearson Correlation Coefficient test shows that both parameters, the visual assessment and the OP-power calculation, are related to the severity of the diabetic retinopathy. When the OP-power and the visual assessment are compared this coefficient (R) equals 0.608 ($p=0.001$), meaning that a positive correlation exists between the categories of visually assessed OPs and the OP-power levels. The total number (N) of subjects which were tested was 108 (27 normal and 81 diabetic subjects). The overall relation of the effect of diabetic retinopathy on OP-power is statistically significant ($p=0.05$). The relationship with the stage of the diabetic retinopathy is not significant ($p=0.19$) for the visual assessment. This means that the OP-power bears more relation to the stage of diabetic retinopathy than is the case in visually assessed OPs. Furthermore, if we compare group 1 of the diabetic retinopathy patients with the reference group no difference is to be obtained by visual assessment, whereas these groups are statistically well-separated by the OP-power (chapter 3.3.2).

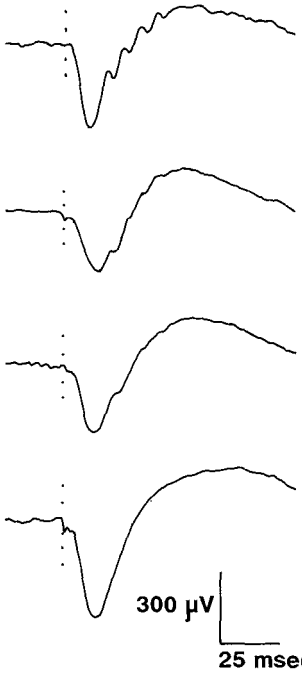


Fig. 4-1: Single flash ERG curve; 1: well developed OPs; 2: lowered OPs; 3: just identifiable OPs; 4: absent OPs

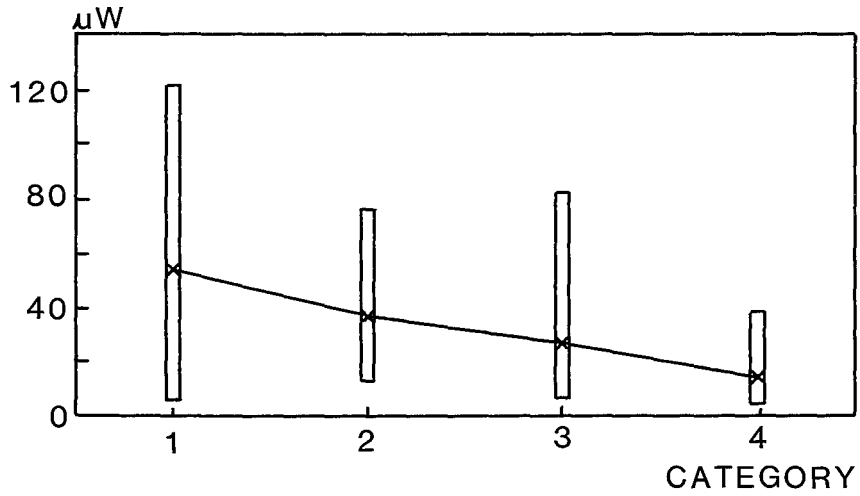


Fig. 4-2: The range in four categories of OPs (open bars) and the mean of each category (x). Horizontal line, category of OPs (table 4-1); vertical line, OP-power in microwatts.

4.1.4 Discussion

In 1972, Galloway et al. found reduced OPs by visual inspection at the established stage of diabetic retinopathy, but failed to prove the existence of a reduction in the non-visible or early stages. This could be due to the rather rough technique which was used. It is above all in these early stages that the improvements in technique to be found in the present study can best be seen, i.e. between the reference group and the first stage and even more between categories 1 and 2. A quantitative measure obviously improves our capacity to differentiate between the OPs.

Using visual inspection the impression was that OP2 is the last one to disappear as the retinopathy increases. This is in compliance with the results described in the second part of this chapter.

4.2 Comparison of amplitude and implicit time of OP2 and OP3 with the op-power

4.2.1 Introduction

The OP-power is a measure for the summed OP activity. The summation of the OP amplitudes is a generally accepted method for the expression of the OP activity in diabetic retinopathy (Algvere, 1968; Miyake and Solish, 1978; Brunette and Lafond, 1983; Bresnick et al., 1984 and Arden et al., 1986). Recently, however Coupland (1987a) as well as Bresnick and Palta (1987a) have considered once again whether the relationship between the amplitude reduction of the second OP and the degree of diabetic retinopathy is stronger than the relation with the summed amplitudes. In this chapter the OP-power, acting as a measure for the summed OP activity, will be compared with the amplitude of OP2 and OP3 separately. Since the implicit time was the most sensitive parameter in detecting early diabetic retinopathy in the study of Okumura (1987), a comparison with this component will also be made.

Since the separate OPs were hard to recognize when the amplitudes were substantially reduced in advanced retinopathies, only the results of the early diabetic retinopathies belonging to groups 1 and 2 are considered in this paragraph.

4.2.2 Method

The diabetic patients are those of groups 1 and 2 as described in chapter 3. The measurements were carried out on the original FIR filtered recording which was made possible by retrieving the original signals from a computer disc. This means that the conditions for ERG recording and signal processing were identical as performed in chapter 2.

From the FIR filtered signal the amplitudes were immediately calculated on the computer's signal processing program (figure 4-3). The implicit times were measured by means of automatic top detection (chapter 2.2.1).

4.2.3 Amplitudes of OP2 and OP3

The amplitudes of OP2 demonstrate a large variance ranging from 31 uV to 169 uV for the diabetic group and from 43 uV to 194 uV for the

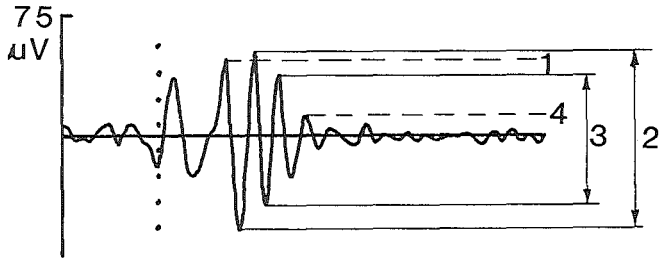


Fig. 4-3: Filtered signal (FIR) of normal single flash ERG of 10 J. 2) amplitude of OP2 being 108 uV , 3) amplitude of OP3 being 79 uV.

Table 4-2. Aplitude of OP2 and OP3

	OP2				OP3		
Diabettic class.	range	SD	mean	N	range	SD	mean
Normal subjects	43-175	32.8	96.0	27	12-125	29.3	59.3
Group1	37-133	26.5	79.9	29	5- 88	24.3	48.1
Group 2	26-153	30.8	75.8	29	6-113	25.2	42.8

reference group (table 4-2). This means a considerable overlap in the case of both ranges. A similar variability is seen for OP3.
The bar diagrams of figures 4-4 and 4-5 represent the distribution into classes of the amplitudes of OP2 and OP3 for the diabetic groups (1 and 2) together with those of the reference group. The amplitudes of the OP2 and OP3 decrease before the appearance of ophthalmoscopical visible diabetic retinopathy, but this decrease is less marked than the attenuation of the OP-power in the early stage of diabetic retinopathy (see also figure 3-4).

The relation between the different OP components, viz. the amplitudes of OP2, OP3 and the OP-power, was tested with the non- parametric version of the Spearman Rank Correlation test (Glanz 1987). Statistically

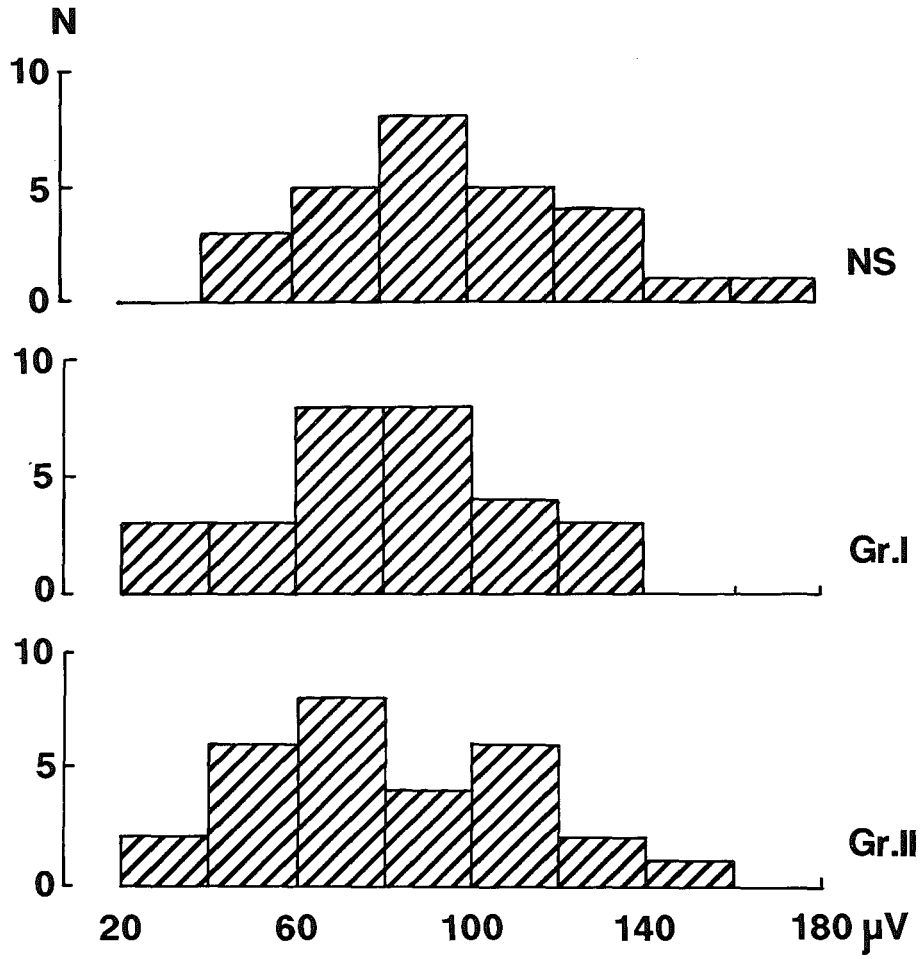


Fig. 4-4: Distribution in classes of the amplitude OP2 in microvolts. NS = normal subjects, Gr.I = diabetic group 1, Gr.II = diabetic group 2.

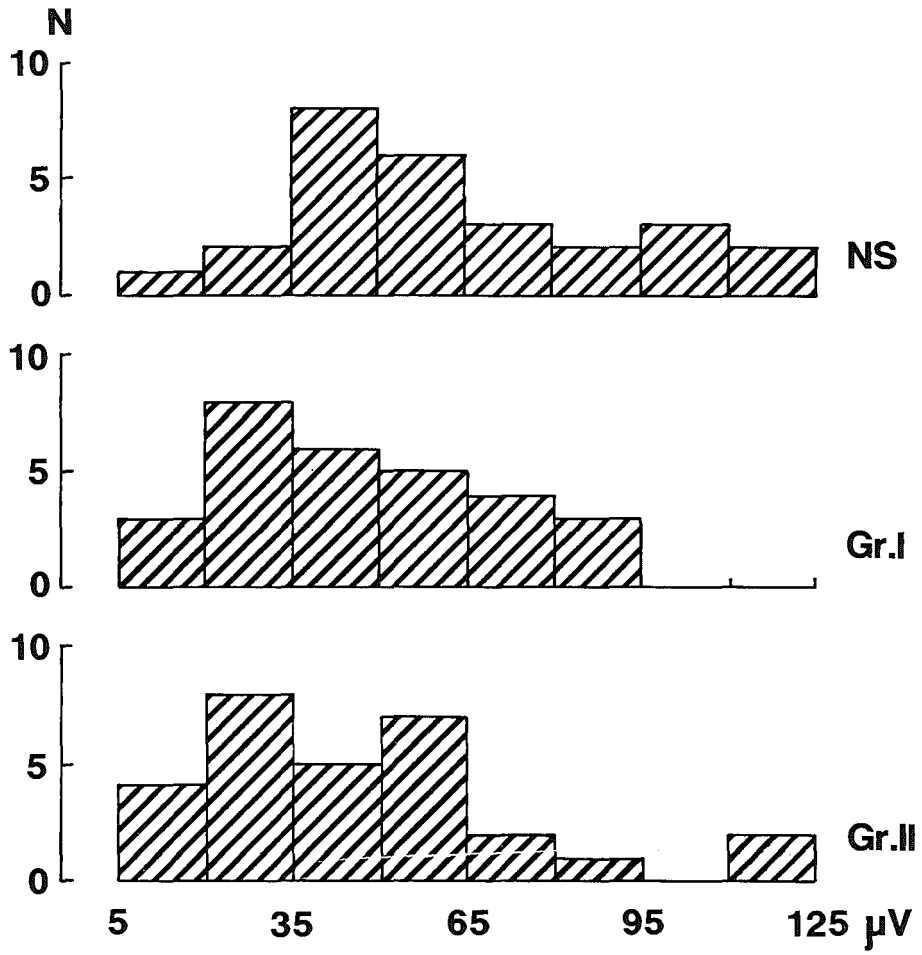


Fig. 4-5: Distribution in classes of the amplitude OP3 in microvolts. NS = normal subjects, Gr.I = diabetic group 1, Gr.II = diabetic group 2.

significant correlations are to be obtained for the right and left eye between OP2 respectively OP3 and the OP-power at the one percent significance level. The correlation coefficients are equal at 0.79. This value means that the three parameters have a good correlation but are not mutually substitutable. Possibly, diabetic retinopathy has a slightly different influence on the separate indicators. For this purpose the effect of the diabetic classification on the amplitudes of OP2 and OP3 was tested with a repeated measurement ANOVA, similar to what was done with the OP-power results in chapter 3.3.2. The overall relation between the three groups (the reference and the two diabetic groups) and the amplitude of OP2 is not statistically significant (p= 0.057). The overall relation for the OP3 of the same three groups is slightly better (p=0.039). This better relation can be primarily attributed to the difference between the reference group and the diabetic group 1 (p= 0.015). However, the OP-power remains an even better measure (chapter 3.3.2).

4.2.4 Implicit time of OP2 and OP3

Measurement of the implicit times of OP2 and OP3 reveals a slight increase for both OPs in relation to early diabetic retinopathy (diabetic group 1 and 2). The effect on the implicit time of both OPs is tested with the non-parametric Krusal and Wallis one-way ANOVA. The p-value is 0.003 for the increase in implicit time of OP2 and 0.002 for OP3 respectively. This increase is highly significant and not surprising in the light of the very small standard deviation (SD) (table 4-3).

The comparison between the effect of the stage of diabetic retinopathy on the OP2 and OP3 implicit times and the effect on the OP-power was

Table 4-3. Implicit time of OP2 and OP3

	OP2			OP3	
Diabetic class.	Mean msec.	SD	N	Mean msec.	SD
Normal subjects	25.3	1.0	27	31.8	1.1
Group 1	26.3	1.4	29	32.8	1.5
Group 2	26.6	1.3	29	33.3	1.6

tested using a statistical model for multiple regression. The dependent variables are the OP-power, the implicit time of OP2 and the implicit time of OP3; the independent variable is the degree of diabetic retinopathy. Table 4-4 presents the estimates. The value of the Adjusted R-square is marginally higher for the implicit time of OP3. The significance level for the OP-power remains the best; for all three, however, they are highly significant ($p << 0.002$).

4.2.5 Discussion

In 1987a Coupland reported a component specific OP reduction in early cases of diabetic retinopathy, from a study which was controlled by fundus photography. This paper and that of Bresnick et al. (1987c) raised our interest in the separate OP parameters and especially in a comparison of them with those of the summed OPs as expressed in the OP-power. The aim of this chapter is to compare the outcome of the separate OP components with that of the OP-power. The Spearman rank correlation coefficient presents a good descending order between the OP-power and the amplitudes of the separate OPs. The results of both techniques reflect the retinopathy state. However, the effect on the OP-power reduction, is more distinct than the effect on the amplitude reduction of the separate OPs. The slight increase in the amplitude of OP2 (not statistically significant) for the diabetic group 2 may be understood by a shift in energy of the OP activity between the separate OPs. This effect was recently suggested by Lachapelle (1988), who studied the possible fusion of the first and second OP and called this form of OP analysis "intensity coding". A higher resistance of the second OP with the other OP wavelets already deteriorating is a more probable explanation for the different behaviour of this component. This OP resistance has also been put forward by Coupland (1987b) and Young et al. (1989) quite recently and is highly dependent on stimulus conditions and adaptation levels. Especially the light adapted OP components reveal these type of changes (Kothe et al., 1989). An explanation for the difference in resistance to the retinopathy of the OP components could be that the OP1 and OP2 represent more the cone activity and the OP3 and OP4 the rod activity (Coupland, 1987b and Aylward, 1989). From the present experiments it appears that the measurement of all the OPs together probably gives a better representation of the functional situation than the isolated OP2 or OP3 component. The explanation may be that the OPs as registered in the present study are predominantly scotopic and the rods are mainly affected in early diabetic retinopathy (chapter 3.1.2).

Table 4-4. Estimates of regression model

	Implicit time OP2	OP-power	Implicit time OP3
Adjusted R square	0.17	0.18	0.22
regression coef/group	+0.02	–0.32	+0.02
t value	+3.1	–3.6	+3.4
level of signific.	< 0.0023	< 0.0005	< 0.0011

The slight increase in implicit time of the OPs in early diabetic retinopathy has already been observed by Yonemura and Kawasaki (1979). Later on, this could not be confirmed by other investigators (Carr and Siegel, 1982; Coupland, 1987b). Recently Okumura (1987) found that an increased implicit time is an earlier symptom than the summed amplitude reduction. In the majority of the diabetic patients in his study this increase was more distinct for the OP1 and OP2 than for the OP3 and OP4. In the present study the increase in implicit time is found to be statistically significant for OP2 and OP3 as well. The effect on the implicit time of OP2 appears to be slightly less significant than the effect on the OP-power (table 4-4). As the increase in implicit time is very small, it may be difficult to investigate this time difference. A high resolution and precise implicit time measurement technique will be necessary for the studying of these small differences. Recently Kobayashi, Shinzato and Yokoyama (1987) put forward some recommendations for arriving at just such an improvement. These authors stated that the latency measurement should be performed following Fourier transform rather than analog filtering procedures. The latter causes a time shift of the entire ERG signal, which is dependent on a- and b-wave magnitude.

In conclusion, it appears that the amplitude measurement provides clinical evidence to support the assumption that the second OP is more resistent to an increase of diabetic retinopathy. Given that the OPs are detected by means of a high resolution technique, the implicit time of the OPs seems to be just as discriminative in detecting early diabetic retinopathy as the OP-power itself.

CHAPTER 5

GENERAL DISCUSSION ON THE OP-POWER MEASUREMENT

The name of the thesis "OP-power measurement in diabetic retinopathy" suggests the existence of a physical measure for the energy of the OPs. As the detailed localisation of the OPs and their origin are not completely understood (chapter 1.7), an absolute scientific validation of the OP measure can not be presented. The measurement interval as has been introduced in chapter 2.2, is more or less arbitrary and the specific OP frequencies can always hide some energy produced by the other ERG components or artefacts.

The term "OP-power" was chosen for the energy calculations, since the term FFT-magnitude, which is sometimes used in cardiological literature, does not refer to the electrical origin but merely to the processing procedure of the signal (Cain et al., 1984).

The aim of the study was to outline the role of the OPs in diagnosing diabetic retinopathy and their relation to standard ophthalmological methods. As early as 1966 Simonson had already reported on a relation between background retinopathy and the disappearence of the OPs. Many observations concerning this relation have been published ever since (chapter 1.6). The conventional method for OP detection proceeds from the individual wavelets by visual assessment of the wavelets or by measurement of the amplitudes and sometimes of the implicit times.

5.1 The single flash OP recording

As the origin and the properties of the OPs are still matters under discussion (chapter 1.7), it is not surprising that many methods have been proposed for the detection of OPs. They differ greatly in their specifications for stimulus luminance and stimulus interval. Stimulus conditions are usually chosen with an intention to obtaining the largest possible OP responses. Wachtmeister recommended a stimulation technique, based on her work carried out in 1974 on luminosity functions of the OPs. She used a luminance whereby both scotopic and photopic functions reach their maximum in the consolidated response (Wachtmeister, 1974b). This can also be achieved by applying conditional flashes or by a 4 Hz flicker (Wachtmeister, 1987).

The multitude of stimulus techniques makes it difficult to compare results. This lead to a recommendation for standardisation by the ISCEV

(International Society for Clinical Electrophysiology of Vision). A standardisation committee was installed and provided its report in 1989.

Basing their work principally on Wachtmeister (1987) the committee advised an averaged 4 Hz white flash ERG in the dark- adapted state. Standardisation increases the possibility of comparing the results between various laboratories, but restricts the possibility of adjusting the technique to certain pathological conditions. Since in the case of diabetes functional loss in the scotopic system is one of the earliest signs of retinopathy, specific conditions are required to enhance the rod contribution (chapter 3.1.2). Therefore, single flashes in the dark were applied and the dark period between the stimuli was increased to three minutes.

Methods to separate cone and rod dominated OPs are still under discussion (King Smith et al., 1986). Various clinical entities probably determine the stimulus conditions required, which means there will be some variation. It is too early yet to make definite choices.

The availability of a computerised system worked as a favourable condition, since this facilitated the conversion of the original ERG signals into a digital signal, which can be processed and analysed in various ways afterwards (chapter 2). When searching for a quantative measure for the OPs, it was sometimes difficult to actually recognize the individual wavelets which often lead to conventional measurement techniques becoming unreliable. Therefore, it is not surprising that many authors have used the summed amplitudes of the individual wavelets as a measure for OP activity (chapter 4.2.1). But even then the individual wavelets have to be recognized and measured.

The OP-power method is based on the principle that the separate OP wavelets have the same origin (Heynen et al.,1985). This was the latest hypothesis in 1986 when the present investigations were started. However, just recently some doubts about this assumption have been expressed (chapter 5.3).

The OP-power appeared to reflect the degree of diabetic retinopathy in a reasonable way and was better than the earlier methods. For the first time abnormal responses could be established before the appearance of an ophthalmoscopically visible retinopathy.

When later on, changes in the separate wavelets became of interest (Bresnick and Palta, 1987c; Coupland, 1987b; Okumura, 1987), the digital storage of the original ERG signals made it possible to analyse OP2 and OP3 separately. Just by retrieving the original signals, a second measurement procedure could be performed (van der Torren et al., 1987; chapter 4-2).

5.2 OPs in the diagnosis and treatment of diabetic retinopathy

In general OPs are not used to establish a diabetic retinopathy or the degree to which it has developed. Ophthalmoscopy and fundus photography are usually sufficient except in the early stages (Moss et al., 1985). Even better than fundus photography is fluorescein angiography (Haut et al., 1987). Ophtalmoscopy, fundus photography and fluorescein angiography all provide morphological information (Nielsen, 1982). The functional state can be examined by standard visual function tests or by specific tests such as detailed perimetry (Bell and Feldon, 1984) and electrophysiology. Electrophysiological examination became of interest after discovering the sensitivity of the OPs for diabetic retinopathy (Yonemura et al., 1962; Simonsen, 1966). When fluorescein angiography cannot be applied or gives insufficient information OP detection becomes all the more important. This is the case in the early stage of diabetic retinopathy, in the blurring of the media (cataract) and in the systemic side effects of intravenous administration of fluorescein (Yannuzzi et al., 1986). A disadvantage with OP registration, for clinical purposes as well as for scientific studies, was the absence of a reliable and quantitative measure. By processing the ERG signal such a measure (OP-power) was achieved.

The OP-power is expressed in a numerical value which allows mathematical analysis. This is especially important in scientific investigations comparing groups. Unfortunately, the large range of the OP-power as recorded makes it difficult to compare individual patients in a normal population, which limits its clinical application. Longitudinal studies using the same individual are certainly feasible.

The large scale and reproducibility of OP-power makes this method especially applicable in early and/or serious retinopathies where other methods fail. As far as the serious retinopathies are concerned, Hennekes

and Deschner (1984) reported a possible increase in OP activity following extensive laser coagulation treatment.

The reproducibility was better under scotopic rather than under photopic conditions. In this regard the mesopic range as advised to obtain high OPs, is a very critical level. Kothe, Lovasik and Coupland (1989) are of the opinion that an unstable adaptive level may possibly be the cause of the fluctuations they found on repeated measurement. They also mention non-uniform retinal illumination and varying electrode impedance as the possible causes of the variability in OP amplitudes. In the discussion they speculate that fluctuations in dopamine release in response to light adaptation may be of influence. Their final conclusion was that implicit time measurements are preferable in longitudinal studies. This conclusion could not be confirmed in the present study when the OPs are registered in the scotopic range (chapter 4.2.4).

Apart from diabetic retinopathy the OP-power may also be helpful in other post-receptoral disturbances for a quantification of the defect. The method has already been succesfully applied for tracing carriers of congenital stationary night blindness (Young et al.,1989).

5.3 Neurogenic function in diabetic retinopathy

Most probably OPs reflect the neuronal activity of the bipolar cells, although the interplexiform cell cannot be excluded as a generator (Wachtmeister, 1987). They may carry information about the functional integrity of the neural elements at the inner retinal layers, while, fluorescein angiography and fluorophotometry give information about the morphologic changes and the vascular function. There is even accumulative evidence that increased permeability, measured by fluorophotometry, is one of the earliest detectable changes in diabetic retinopathy (Cunha-Vaz, 1981). Therefore, it would be interesting to compare the fluorophotometry together with the fluoroangiography as measures for capillary dysfunction to the OP-power in early diabetic retinopathy, in order to find out, whether the neural changes will coincide with a dysfunction of the blood retina barrier. Yoshida et al. (1987) has already carried out a comparative study between OP measurement and fluorophotometry. He concluded that the implicit time of the OPs was increased at an early stage of diabetic retinopathy. The implicit time was even earlier disturbed than other parameters such as fluorophotometry

and OP amplitude. There is a likelihood of the neuronal dysfunction being caused by a direct defect in the bipolar cell layer, in the sense that it is possibly osmotic, rather than by ischaemia of this cell layer. If this is the case vascular dysfunction and neurogenic dysfunction does not have to decrease at the same time. The early decrease of OP-power in diabetic retinopathy might prove to be an argument in favour of such a theory.

Another argument pointing in the same direction comes from the morphometric analysis of retinal blood vessels in diabetic retinopathy. Fuchs et al. (1985) concluded from their study that pericyte loss and crude capillary damage cannot be the primary cause of neuronal degeneration, since the pericytes disappear at the same time and in the same pattern as the other extravasal cells.

As already mentioned OP-power is based on the principle that the OPs originate in the same structure (Heynen, 1985). Later investigators propose the reverse namely that it is not the bipolar cells which generate the OPs, but that the OPs are generated between the inner and outer plexiform layers by synaptic feedback circuits (Yanagida et al., 1988). It is even questionable whether the increase in implicit time and the decrease in amplitude are caused by the same mechanism, since Yonemura and Kawasaki (1979) demonstrated that quinaldic acid affects the implicit time of the OPs selectively. This is in accordance with the recent finding of Sannita et al. (1988) This author describes a direct effect of neurotransmitters as L-acetylcarnitine on the implicit times of the OP2 component, which is that of causing a significant reduction. He did not find statistically significant changes in the amplitude of any of the separate OPs.

This finding could not be confirmed in the present study. In early diabetic retinopathy the implicit times of the OP2 and OP3 were delayed at the same stage of the disease as that at which the OP-power was decreased.

The possibility that the second OP is less sensitive to diabetic retinopathy may contradict the conclusion that OPs originate in the same structure (Heynen et al., 1985). Another argument in favour of the hypothesis is that separate OPs do not have the same nature, which can also be deduced from the finding of Marmor et al. (1988). He found that especially the second OP is selectively suppressed by a D1 antagonist in experiments using a rabbit. The present study also indicates a selective resistence of OP2 (chapter 4.2.3). The mechanism is still unknown. However, a study

in which cone or rod dominated OPs are clearly separated might give the answer to the difference in behaviour of OP2 (Coupland, 1987b).

5.4 Other applications of OP measurement in diabetic retinopathy

The neural function may be of interest in testing procedures, regarding the effectiviness and side effects of new drugs.

The factor OP-power may prove its value in situations like those in which groups of diabetic patients are tested for their reaction to newly developed treatment in research protocols.

Another application may be the predictive value of OP reduction when diabetic retinopathy increases towards the proliferative stage. This was reported by Simonsen (1975) and Bresnick and Palta (1987b). Even more important would be the possibility for identification of the preproliferative stage using OP-power. This stage is of special interest since it seems to be the most favourable point for starting coagulation therapy (Little, 1985). The ETDRS (1987) started a study to investigate this matter.

In our classification system the preproliferative stage is represented by group 3. The diagram of figure 5-1 shows a logistic model which describes how the diabetic groups 2 and 3 can be distinguished on the basis of their OP-power. This model is estimated from the OP-power measurement of the left eye of patients in groups 2 and 3. In these patients (26 patients in group 2 and 15 patients in group 3) the break-even point is at 13.8 microwatt, where the probabilities of belonging to group 2 or to group 3 are the same (50%). The chance for getting serious ischaemic changes in the retina has to be considered below the 13.8 microwatt level, where the probability of belonging to group 3 is larger than the probability of belonging to group 2. This may have consequences for the timing of the photocoaculation treatment.

With the development of personal computer technology the costs, for special methods like OP-power measurement, will decrease and will become in reach of many clinical laboratories. In particular the storage of ERG responses has been improving since the memory of the hardware has started to expand (Salu et al., 1989). This technical development can be used for longitudinal studies in diabetic retinopathy.

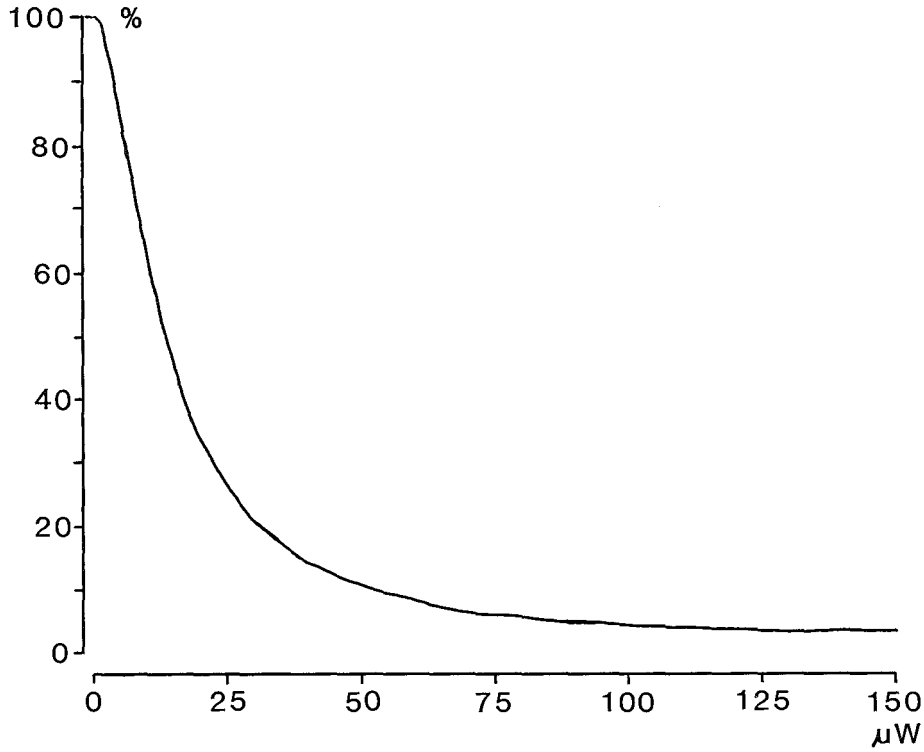


Fig. 5-1: A logistic model representing the discrimination between groups 2 and 3; it expresses the probability of belonging to group 3 as a function of OP-power. Horizontal line: OP-power in microwatts; vertical line: probability (%) of belonging to group 3.

SUMMARY AND CONCLUSIONS

The present study is mainly concerned with the role of the OPs in diagnosing early diabetic retinopathy. In 1966 Simonsen had already found a clear relation between background diabetic retinopathy and the disappearance of OPs at the ascending limb of the b-wave of the ERG. Many investigators have studied this relationship ever since, but because of a deficiency in a quantification measure of the OPs, the clinical application has been unsatisfactory. Recent developments involving digitalising of the ERG signal and the accompanying related increase in signal processing techniques, nowadays offer us the opportunity of developing a reliable system of OP quantification.

Diabetic retinopathy is characterized by multiple vascular lesions of the eye fundus. The clinical course is quite variable, but one or another feature may predominate the fundus picture at a given time. The knowledge of the pathogenesis in diabetic retinopathy remains incomplete as yet; as does the precise cell localisation of OPs and their relation to diabetic retinopathy. As long as this knowledge is incomplete a classification and measurement system of the retinal function in this disease will be defective.

In chapter 1: a review has been given of the literature containing theories concerning the pathogenesis of diabetic retinopathy, as well as the history and origin of the electroretinographic features.

Early salient features in diabetic retinopathy are vasodilatation and microaneurisms together with capillary closure. These early changes are important as the aim of this study is to identify the role of the small wavelets known as oscillatory potentials (OPs) on the ascending b-wave of the ERG.

Chapter 2 concerns the detection methods of the OPs and explains the advantages of a digital system above an analog filtering system. Sometimes it was difficult to interpret the results of the digitally filtered signal. Thereupon, an effort was made to improve the measurement method by signal processing. This resulted in a new clinical method in

which a digital filter was applied to the single flash 10 J ERG response followed by Fast Fourier Transform (FFT).

The frequencies of the OPs and those of the foregoing a-wave are sometimes in the same range. The main problem was their separation.

The energy of the OPs is calculated by determining the dominant frequency around which a floating window specifies the frequency domain. The calculated energy is represented by the OP-power and is expressed in microwatts. As the digital filter does not cause a phase shift, the filtering program can be extended by an automatic top detection in order to measure the implicit times and to calculate the amplitudes of the OPs.

In chapter 3 a clinical study is described concerning the OP- power measurement in four groups of diabetic patients. The diabetic retinopathy varies from a non-visble towards a preproliferative stage. Special attention has been paid to the features of diabetic retinopathy group 1. The question was at which stage of diabetic retinopathy could electrophysiologic abnormalities be detected. The OP-power is measured under scotopic conditions. The duration of the stimulus interval has been discussed with reference to the literature. The frequency shift to higher values when the stimulus interval is increased, positively influences the identification of the frequency domain of the OPs.

The overall effect of diabetic retinopathy on the OP-power is statistically highly significant. The decrease in OP-power, when the retinopathy group 1 is compared with the reference group, is found to be statistically significant as well. From this it may be concluded that using the OP-power measure diabetic retinopathy can be detected at an early stage. In the study, the OP-power method does not provide sufficient information under photopic conditions. This may be due to the relatively large decrease in the frequency of the OPs in the light adapted state. The OP-power as applied under scotopic conditions appears to have a stable reproducibility, which is in contrast to a recent study by Kothe et al. (1989) concerned with OPs under photopic conditions. The dominant frequency of the FFT in the domain at which the OP activity is to be suspected scotopically, shows a shift to lower frequencies while the stage of retinopathy increases to the preproliferative stage.

In chapter 4-1 a comparison has been made between OP-power recordings

and visual assessment of the OPs. The OP-power method revealed a better relation with the diabetic retinopathy statistically than did the subjective method, although there is a good correlation between the two methods.

In chapter 4-2 a comparison was made between OP-power and the amplitude of the separate wavelets OP2 and OP3. The reduction in amplitude of the separate OP2 wavelet is not statistically significant. The OP3 wavelet, however, provides a "just- significant" reduction, while the decrease in OP-power is "highly significant" ($p < 0.001$). The increase in the implicit time of the OP2 and OP3 appears to be significant for the diabetic groups 1 and 2. This increase is nearly as effective in reflecting the stage of the diabetic retinopathy as the OP-power is.

Chapter 5 contains a general discussion. A few ideas are formulated to achieve the OP-power method in future studies.

In conclusion, it may be stated that the results of the OP-power method show a convincing improvement in the methods for the detection of electroretinographic changes in early diabetic retinopathy. The OP-power results are better when compared to the conventional techniques as well as when compared to the individual results of the wavelets OP2 and OP3.

The OP-power method substantiates a quantative measure of the OPs even in advanced stages of diabetic retinopathy.

SAMENVATTING EN CONCLUSIE

Het doel van deze studie is na te gaan welke plaats de Oscillatory Potentials (OPs) inneemt als diagnostische onderzoekmethode tussen de functieonderzoeken die bij diabetische retinopathie in gebruik zijn. In 1966 ontdekte Simonsen reeds dat er een duidelijke relatie bestaat tussen het ontstaan van achtergrond diabetische retinopathie en het verdwijnen van de OPs op de stijgende tak van de b-golf in het ERG. Sindsdien zijn er vele onderzoekers geweest die zich met deze relatie bezig hebben gehouden. Wegens gebrek aan mogelijkheden tot kwantificering van de OPs waren de uitkomsten van deze onderzoeken, zeker voor klinische toepassing, nog onbevredigend. De recente ontwikkeling in het digitaliseren van ERG signalen en de daarmee samengaande mogelijkheden tot signaalbewerking, stelt ons thans in de gelegenheid om een betrouwbare kwantificering van de OPs te ontwikkelen.

Diabetische retinopathie wordt gekarakteriseerd door multipele vasculaire laesies van de fundus, waarvan het klinische beeld sterk varieert. Bepaalde verschijningsvormen kunnen echter op een zeker tijdstip het beeld domineren. Het mechanisme dat leidt tot de pathogenese is nog onvolledig bekend, evenals de oorsprong van de OPs op cellulair niveau en hun relatie tot de diabetische retinopathie. Zolang de kennis van de pathogenese onvolledig is, zal elk classificatiesysteem en functieonderzoek te kort schieten.

In hoofdstuk 1 worden uit literatuurstudie verkregen theorieën over de ontstaanswijze van diabetische retinopathie beschreven. Eveneens wordt een historisch overzicht gegeven over het electroretinografisch beeld van de diabetische retinopathie en over de oorsprong van de OPs. Vasodilatatie en microaneurysmata zijn, samen met capillaire afsluiting, de meest opvallende vroege kenmerken. Deze vroege veranderingen zijn belangrijk, daar het doel van deze studie is de rol van de OPs in het ERG tijdens dit stadium van de ziekte te onderzoeken.

Hoofdstuk 2 behandelt de detectiemethoden voor OPs en laat de voordelen zien van een digitaal boven een analoog filtersysteem.

In sommige gevallen was het moeilijk om de resultaten van het digitaal gefilterde signaal te interpreteren. Vervolgens werd getracht de meetmethode te verbeteren met behulp van signaal bewerking. Dit resulteerde in de invoering van een nieuwe klinisch toepasbare meetmethode, waarbij de 10 J enkele flits responsie digitaal gefilterd werd, gevolgd door Fourier analyse van het gefilterde signaal.

De frequenties van de OPs en die van de daaraan voorafgaande a-golf liggen soms in hetzelfde gebied. Het belangrijkste probleem was deze te scheiden. De energie van de OPs wordt berekend door de dominante frequentie op te zoeken, waaromheen een variabel venster het frequentiegebied aangeeft. De berekende energie wordt weergegeven door de OP-power en wordt uitgedrukt in microwatts. Aangezien de digitale filter geen fase verschuiving introduceert, kan het filterprogramma worden uitgebreid met een automatische top detectie teneinde de piek latentie tijden te meten en de amplituden te berekenen.

In hoofdstuk 3 wordt een klinische studie beschreven betreffende de bepaling van de OP-power bij vier groepen diabetes patienten. De diabetische retinopathie van deze patienten varieert tussen een niet zichtbare en een preproliferatief stadium. Speciale aandacht werd besteed aan de kenmerken van de retinopathie groep 1, om te bepalen in welke fase er elektrofysiologische veranderingen kunnen worden waargenomen.

De OP-power wordt gemeten onder scotopische omstandigheden. De duur van het interval tussen de stimuli wordt aan de hand van een literatuuroverzicht besproken. De frequentie van de OPs wordt hoger bij het toenemen van de interstimulustijd, hetgeen het scheiden van het OP frequentiegebied van de a-golffrequenties verbetert.

Het effect van de diabetische retinopathie als geheel op de OP-power is statistisch sterk significant. Het afnemen in OP-power, bij vergelijking van de diabetische retinopathie-groep 1 met de referentie-groep, is statistisch ook significant. Hieruit kan de conclusie worden getrokken, dat met de OP-powermethode de diabetische retinopathie in een vroeg stadium kan worden opgespoord.

De OP-power geeft onder fotopische condities, zoals in deze studie is toegepast, niet voldoende informatie. Dit kan een gevolg zijn van de sterke frequentiedaling van de OPs bij lichtadaptatie.

De OP-power heeft onder de toegepaste scotopische condities een goede reproduceerbaarheid, dit in tegenstelling tot de OP bepalingen in de recente studie van Kothe en medewerkers (1989) onder fotopische condities.

De dominante frequentie van het frequentiegebied, waarin de OPs scotopisch verwacht worden, neemt af bij het toenemen van de retinopathie naar een preproliferatief stadium.

In hoofdstuk 4-1 wordt een vergelijking gemaakt tussen de beschreven OP-power en de visuele beoordeling van de OPs. De relatie tussen de OP-power en de diabetische retinopathie bleek statistisch beter gedefinieerd dan die van de visueel beoordelde OPs, ofschoon er een goede correlatie bestaat tussen de twee methoden.

In hoofdstuk 4-2 wordt OP-power vergeleken met de uitkomsten van de afzonderlijke amplituden OP2 en OP3. De reductie in amplitude van de OP2 is statistisch niet significant. Die van de OP3 is net significant, terwijl de afname van de OP-power statistisch sterk significant is ($p < 0.001$). Het toenemen van de piek latentie-tijden van de OP2 en de OP3 blijkt eveneens significant te zijn voor de diabetische retinopathiegroepen 1 en 2. Deze toename is bijna even effectief in het weergeven van het groepseffect als de OP-power.

Hoofdstuk 5 bevat een algemene discussie. Enkele suggesties voor de toepassing van de OP-power methode bij toekomstige studies worden er gegeven.

Concluderend kan worden gesteld dat de resultaten van de OP-power methode een overtuigende verbetering zijn in de bepaling van de elektroretinografische veranderingen bij vroege diabetische retinopathie. De resultaten met de OP-power zijn beter in vergelijking met die van de bestaande technieken en die van de afzonderlijke amplituden van OP2 en OP3.

De OP-power geeft een kwantitatieve maat aan de OPs, zelfs in gevorderde stadia van diabetische retinopathie.

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CURRICULUM VITAE

De schrijver van dit proefschrift werd op 4 september 1948 te Geleen geboren. Hij deed eindexamen HBS-B aan het Nederlandsch Lyceum te Den Haag in 1968. Aansluitend studeerde hij Geneeskunde aan de Rijksuniversiteit te Leiden. Van 1970 tot 1972 was hij werkzaam als studentassistent op het Laboratorium voor Stollings Physiologie onder leiding van wijlen Dr. J.J. Veltkamp.
In 1975 werd het artsexamen behaald.
De militaire dienst werd in de periode 1975/1976 vervuld op de afdeling oogheelkunde van het Militair Hospitaal te Utrecht.
Op 1 oktober 1976 begon hij zijn opleiding tot oogarts in het Oogziekenhuis te Rotterdam (opleider: Prof. Dr. H.E. Henkes). Op 1 oktober 1980 werd hij ingeschreven in het Specialisten Register als oogarts.
Sinds juli 1981 is hij als oogarts verbonden aan het Merwede Ziekenhuis te Dordrecht, alwaar hij sinds 1986 het onderzoek beschreven in dit proefschrift heeft verricht onder leiding van Prof. Dr. G.H.M. van Lith.