
Age-related maculopathy: a multifocal approach

Thesis submitted by

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MD

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Keywords

Age-related maculopathy, ARM, age-related macular degeneration, AMD, early ARM, late ARM, cone-mediated multifocal electroretinogram, rod-mediated multifocal electroretinogram, mfERG, subjective vision measures, psychophysical tests, objective vision measures, photodynamic therapy, PDT.

ABSTRACT

Age-related maculopathy (ARM) is a central retinal disease with unclear pathogenesis. It is the major cause of permanent vision loss in adults over 50 years and is increasing in prevalence and incidence, faster than the aging population would suggest. Early in the disease process (early ARM) there is little or no vision loss and there are only slight retinal changes with abnormal deposits within Bruch's membrane. As the disease progresses (late ARM or age-related macular degeneration, AMD) vision loss may be quite severe due to atrophy (dry AMD) or the development of chorioretinal neovascularisation (CNV, wet AMD). It is hard to predict from conventional eye examinations and clinical vision tests which cases will progress to the severe, dry or wet forms of the disease. Moreover, most of the conventional clinical tests are based upon subjective vision measures. Objective tests which detect ARM earlier would be a useful aid to diagnosis and to monitoring progression.

The multifocal electroretinogram (mfERG) is a relatively new clinical tool which enables the recording of electrical potentials from multiple, small areas of the central retina and thus assesses function from specific retinal locations. It is therefore useful in detecting focal retinal diseases such as hereditary or acquired maculopathies or in monitoring retinal laser or surgical treatment effects.

There is cone and rod impairment in ARM and histopathological and psychophysical evidence for a preferential vulnerability of rods compared to cones. This research project investigated if an objective tool such as the mfERG could detect early ARM,

its progression and the treatment effects of multiple photodynamic therapies (PDT) on retinal function in late ARM, prior to a battery of subjective vision measures.

For comparison purposes a subjective assessment of central retinal function was performed using high and low contrast distance visual acuities (VA), near VA, low luminance VA (SKILL cards), contrast sensitivity (Pelli-Robson, P-R), saturated and desaturated Panel D-15 (sat Panel D-15, desat Panel D-15) and central visual fields (Humphrey 10-2, mean sensitivity, MS and mean defects, MD). As an objective assessment of central retinal function the cone- and rod-mediated multifocal electroretinograms were recorded.

Subjective and objective tests of retinal function were compared in early ARM and an age-matched control group (chapter 3). Seventeen eyes of seventeen subjects with early ARM and twenty control subjects with normal vision were measured. For the cone-mediated mfERG responses conventional averaging methods were used and results were correlated with subjective vision tests. The conventional cone-mediated mfERG failed to distinguish between the early ARM and control subjects whereas subjective vision measures such as HC- and LC-VA, desat Panel D-15, MS, P-R were significantly reduced in the ARM group. However, there were significant correlations between the cone-mediated mfERG and the desat Panel D-15 results in the ARM group. This suggests that the mfERG measures similar retinal processes that detect colour vision deficiency under desaturated conditions. There was no significant correlation between cone-mediated mfERG measures and funduscopy changes. The conclusion from this study was that the subjective vision tests detected early ARM better than the objective cone-mediated mfERG. Thus the aim of

detecting early ARM objectively was not met by the cone-mediated mfERG suggesting the need to develop other objective tests such as a rod-mediated mfERG.

Whether the preferential rod vulnerability others have reported in early ARM could be detected by the rod-mediated mfERG was determined in the next study (chapter 4). A protocol for recording rod-mediated mfERG responses was developed by determining the optimal testing luminance to reduce the effect of stray light and elicit maximal rod-mediated responses. Sixteen of the seventeen ARM subjects and seventeen control subjects from the previous study were tested. For analysis, a customized computer template fitting method was developed in MATLAB (Mathworks, Natick, MA, USA). This method has been shown to be useful for low signal-to-noise ratio responses that characterize the rod-mediated mfERG. Significantly delayed rod-mediated mfERG responses were found whereas cone-mediated mfERG responses were within the normal range. This suggested that the effect of ARM on the rod system could be detected objectively with the rod-mediated mfERG before changes in the cone-mediated mfERG.

Which of the tests best detected progression of vision loss was investigated in chapter 5. Visual function of 26 (13 ARM and 13 control subjects) of the original 37 subjects (17 ARM and 20 control subjects) had cone- and rod-mediated mfERG and the subjective vision measures repeated after one year. The main purpose was to determine which of the tests best detected progression of vision loss. The mfERG results were analysed by using both averaged and local responses and by using the computer template fitting procedure. On average no significant worsening of either objective or subjective function measures was evident after one year. These results

reinforce the slow progression of the disease. With a longer follow-up period progression of ARM may translate into measurable changes in the mfERG and the other visual function tests.

The effect of multiple photodynamic therapies (PDT) on cone- and rod-mediated function was assessed with the mfERG in the last study (chapter 6). The cumulative treatment effects of PDT in five subjects with late ARM were determined. Having demonstrated that the rod-mediated mfERG was applicable in early ARM, this study also aimed to investigate how useful it was in late ARM where there is substantially greater rod loss. Cone- and rod-mediated mfERGs, visual acuities, contrast sensitivities and central visual fields were investigated a week before treatment began and then one month after each PDT treatment. The subjects received three treatments each over an average period of five and a half months. In some subjects there were significant transient reductions in cone- and rod-mediated amplitudes possibly reflecting alterations in choroidal hypoperfusion dynamics one month after treatment. Further, b-wave component of the mfERG became increasingly misshapen after each PDT treatment suggesting an ischemic insult mainly targeting post-receptoral sites. However, objective and subjective function was stabilized after multiple PDT treatments in most of the subjects. This pilot study of five cases showed that there was no additional damage to cone- and rod-mediated outer retinal function after three PDT treatments.

One of the novel findings of this research was that the rod-mediated function measured with the mfERG was impaired in early ARM. This finding supports histopathological and psychophysical evidence of rod vulnerability in early ARM.

The results of these studies also suggest that early ARM affects different aspects of visual function which is reflected by different outcomes from objective and subjective vision tests. A model (chapter 7) based upon the results was developed proposing a hypoxic insult with a preferential alteration of post-receptoral sites in early ARM.

The cone-mediated mfERG documented the retinal damage and possible treatment effects on outer retinal function of the multiple PDTs which did not further deteriorate. Thus, this technique might assist in the development of optimal treatment modalities for ARM, especially in retreatment regimes. Greater variability was found for the rod-mediated mfERG and its clinical use in PDT treatment regimes still needs to be investigated.

In conclusion, this research has provided a better understanding of the disease process and treatment effects in ARM and might contribute to improvements in diagnosis and treatment of ARM.

List of Publications

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STATEMENT OF ORIGINAL AUTHORSHIP

“The work contained in this thesis has not been previously submitted for a degree or diploma at any other higher education institution. To the best of my knowledge and belief, the thesis contains no material previously published or written by another person except where due reference is made.”

Signed:.....

Date:.....

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CHAPTER 1

Introduction

Age-related maculopathy (ARM) is now recognized as the most common registered cause of blindness in the Western world (Ghafour et al. 1983; Leibowitz et al. 1984; Evans and Wormald 1996). The pathogenesis is still unclear and genetic predisposition together with environmental factors have been implicated (Bird 1991; Pauleikhoff 1992; Grunwald et al. 1998; Beatty et al. 2000b; Johnson et al. 2000; De Jong et al. 2001; Friedman et al. 2004b; Holz et al. 2004). Photoreceptor death and vision loss result from subretinal choroidal neovascularisation (CNV) or retinal pigment epithelium (RPE) detachment or from central geographic atrophy (GA, late ARM). These can occur in response to depositions of abnormal material within Bruch's membrane which accumulate during the early course of the disease (early ARM) (Gass 1967; Gass 1973; Sarks 1976; Bird 1991; Boulton 1991; Sarks et al. 1994; Guymer et al. 1998; Boulton and Dayhaw-Barker 2001; Holz et al. 2004).

However, recent histological findings have indicated a loss of rods in the area immediately surrounding the central retina in early ARM (Curcio 2001). In addition studies of dark adaptation in early ARM have suggested that rods are first affected (Owsley et al. 2000; Owsley et al. 2001). In eyes with late ARM the number of rods decreases rapidly while the last surviving photoreceptors are cones (Curcio 2001). This suggests that tests based on rod-mediated functions should detect ARM at earlier stages than tests of cone function and changes should be detected well before visual acuity is affected.

The conventional treatment of the CNV in late ARM involves argon or krypton laser therapy of abnormal retinal blood vessels (Macular Photocoagulation Study Group 1982; Macular Photocoagulation Study Group 1986; Macular Photocoagulation Study Group 1990a). This treatment is only effective in a small percentage of patients with ARM, causes immediate chorioretinal damage and can lead to additional vision loss. Photodynamic therapy (PDT) is more selective by targeting the CNV directly and sparing the overlying neurosensory retina (Schmidt-Erfurth and Hasan 2000; Schmidt-Erfurth et al. 2002a). PDT has been shown to stabilize visual acuity, visual fields and contrast sensitivity in ARM patients (Treatment of Age-Related Macular Degeneration With Photodynamic Therapy (TAP) Group 1999; Treatment of Age-Related Macular Degeneration With Photodynamic Therapy (TAP) Study Group 2001; Palmowski et al. 2002; Rubin et al. 2002; Treatment of Age-Related Macular Degeneration With Photodynamic Therapy (TAP) Study Group 2002; Schmidt-Erfurth et al. 2004). Usually a number of treatments to stabilise vision is required for up to several years after diagnosis of late ARM. Unfortunately, the majority of patients have untreatable disease, whilst the minority who have disease amenable to treatment have a better visual prognosis the earlier treatment is performed (Hopley et al. 2004a; Smith et al. 2004). Treatment decisions are mainly based upon morphological features visible in angiography together with the reduction in visual acuity and are still under debate regarding the optimal number of retreatments and interval between retreatments. Thus in addition to morphological features and subjective vision measures, objective assessment of early functional impairment might be advantageous in setting optimal treatment parameters. Additionally, due to evidence of prolonged choroidal hypoperfusion (Michels and

Schmidt-Erfurth 2003), the effect on outer retinal function of multiple treatments is still not clear and needs further investigation.

As the imaging techniques in retinal diseases have improved, new methods for measuring localized retinal dysfunction such as the multifocal electroretinogram (mfERG) have been introduced (Sutter and Tran 1992). In contrast to the full-field ERG the mfERG measures the retinal function at many local areas of the central retina up to a 50° area in a short examination time (by its conventional use in less than 10 minutes). This has enabled researchers to investigate central retinal diseases in more detail (Sutter and Tran 1992; Parks et al. 1996; Kretschmann et al. 1997; Palmowski et al. 1999b; Seeliger et al. 2001a). Given that ARM is a localized disease and the mfERG measures local retinal responses, the mfERG is a powerful tool for the functional mapping of the central retina in ARM, especially in early ARM where visual acuity is hardly impaired, an objective measure of severity is advantageous.

Psychophysical tests measuring both steady-state as well as kinetic aspects of either cone or rod function have been shown to be sensitive in detecting early ARM (Brown et al. 1986a; Brown et al. 1986b; Eisner et al. 1991; Cheng and Vingrys 1992; Owsley et al. 2000; Owsley et al. 2001; Phipps et al. 2003). Some studies show inconsistent results, for example regarding colour vision defects (Eisner et al. 1991; Midea et al. 1997) or have not found significant correlations with ophthalmoscopic changes (Sunness et al. 1988; Collins and Brown 1989b; Sunness et al. 1989a; Cheng and Vingrys 1992; Tolentino et al. 1994). Progression of early ARM has been described as slow with only 5%-30%, developing the next stage within the following 6.5 years (van Leeuwen et al. 2003b). This might imply the need for objective cone

and rod function tests that can measure early ARM and its progression earlier than subjective tests and funduscopy.

The cone-mediated mfERG appears to be affected to some extent in early ARM although studies are not consistent as to whether amplitude or latency is first affected (Huang et al. 2000; Li et al. 2001; Palmowski et al. 2001; Gerth et al. 2002). This is possibly due to different study designs, some of which have investigated limited numbers of subjects, have used both eyes of each subject or have not used international fundus grading systems and age-matched control groups (Huang et al. 2000; Li et al. 2001; Palmowski et al. 2001; Gerth et al. 2002). Additionally it is known that the mfERG amplitude shows a high variability and age dependency (Fortune et al. 1999; Seiple et al. 2003). Most of the early ARM studies involving the cone-mediated mfERG have used averaging methods and/or analysis methods less sensitive to latency changes (Parks et al. 1996; Huang et al. 2000; Li et al. 2001).

The first study of this thesis (chapter 3) was designed to measure the cone-mediated mfERG under well-controlled conditions which improved on some aspects of previous studies. These conditions included participation of an age-matched control group, grading fundus changes based on an international grading system and including only one eye of each subject in data analysis. It was of interest to determine if the cone-mediated mfERG was impaired prior to reduced vision being detected by clinical subjective vision function tests. Given that correlations between the cone-mediated mfERG and psychophysical measures have been found to be contradictory (Odel et al. 1999; Holopigian et al. 2001; Holopigian et al. 2002; Jurklies et al. 2002; Seiple et al. 2002), subjective vision measures were also

performed and correlated with the mfERG to investigate if these tests were affected differently by ARM. This was based upon the suggestion that the different function tests measure different aspects of vision depending on how a retinal disease affects sensitivity as a function of adaptation level (Seiple et al. 2002).

Given the preferential rod vulnerability before cones in early ARM (Curcio et al. 1996), the next experiment (chapter 4) investigated the rod-mediated mfERG to examine if it was superior to the cone-mediated mfERG in detecting early ARM. For this purpose a rod-mediated mfERG was developed based upon the suggestions of Hood et al. (1998b). Hood et al. (1998b) concluded that optimal stimulus conditions needed to be further investigated, as they based their recommendations on results from two experienced young observers. Thus, another aim in this second experiment was to determine if a rod-mediated mfERG test protocol could be developed for a larger group of older, inexperienced subjects.

The third experiment (chapter 5) monitored visual function of the early ARM subjects after a period of one year. A battery of subjective and objective vision tests was performed at baseline and again after one year. It was hypothesized that the rod-mediated mfERG function would detect changes in retinal function before there was impairment in other function tests or visible ophthalmoscopic progression.

We were interested in how useful the cone- and rod-mediated mfERG were for monitoring treatment effects in late ARM. Cone-mediated mfERGs have been measured in late ARM after only one session of PDT (Palmowski et al. 2002; Jiang et al. 2003; Lai et al. 2004). Given that usually several treatments are necessary, the

cumulative effect of multiple laser therapies on cone and rod-mediated retinal function was investigated in a pilot study (chapter 6). This was especially of interest because surrounding healthy areas have been demonstrated to show substantial hypoperfusion after treatment influencing visual outcome (Schmidt-Erfurth et al. 2002b; Schmidt-Erfurth and Michels 2003).

The first hypothesis of this thesis was that the cone-mediated mfERG would measure changes in function before conventional psychophysical vision tests in early ARM (chapter 3). It was speculated that the rod-mediated mfERG would detect early ARM (chapter 4, hypothesis 2) and its progression (chapter 5, hypothesis 3) earlier in the disease than cone-mediated tests. The last hypothesis was that there would be no additional cone- or rod-mediated dysfunction, either of the treated area or of the surrounding healthy area, measured by the mf-ERG as a result of multiple PDTs (chapter 6, hypothesis 4).

CHAPTER 2

Literature review

2.1 Early and late age-related maculopathy

2.1.1 Definition

According to the International Age-Related Maculopathy Epidemiological Study Group (Bird et al. 1995) age-related maculopathy (ARM) is classified as either early ARM with drusen and retinal pigmentary epithelium (RPE) abnormalities with little or moderate visual disturbances or as advanced, late ARM or age-related macular degeneration (AMD) (Fig. 2.1). The latter form is accompanied by severe vision loss due to a central geographic atrophy (dry AMD) or due to ingrowth of choroidal neovascularisation (CNV) from the underlying choroid (neovascular AMD or wet AMD).

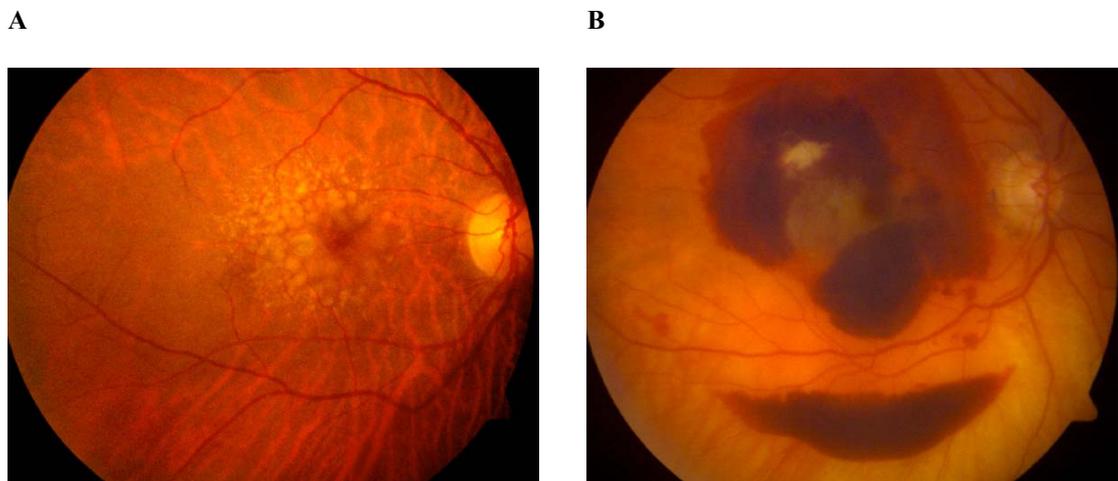


Figure 2.1. The funduscopy features of early ARM with drusen (A) and late, wet ARM (AMD) with a chorioretinal neovascularisation membrane and subpigment epithelial, subretinal and preretinal bleeding.

Funduscopy discrete whitish-yellow spots are identified as drusen ($\geq 63\mu\text{m}$) and are either hard, soft distinct (with uniform density and sharp edges) or soft indistinct (with decreasing density from the centre outwards with fuzzy edges). Smaller hard

drusen ($<63\mu\text{m}$) themselves are not considered to characterize the disorder in some grading systems and thus are not included (Bird et al. 1995) whereas in other grading systems those are considered and graded in the lowest (earliest) levels (Klein et al. 1991; Age-Related Eye Disease Study Research Group 2001c). Retinal pigment abnormalities are either graded as hyperpigmentation or depigmentation whereas geographic atrophy (GA) is defined by a sharply demarcated area, round or oval area of RPE depigmentation $\geq 175\mu\text{m}$. Wet AMD may be made up of CNV, RPE and neurosensory retinal detachment, hard exudates, retinal haemorrhages and intraretinal or subretinal scars and is supposed to be graded according to presence, location and area covered by the lesion.

2.1.2 Prevalence and incidence

Age-related maculopathy (ARM) has become a major public health issue as it is the leading cause of blindness in the older population (>50 years) of the Western world (Leibowitz et al. 1984; Hyman 1992; Evans 1995; Attebo et al. 1996; Evans and Wormald 1996; Weih et al. 2000). For example, it has been demonstrated that registrations for blindness and low vision attributed to ARM have increased in the order of 30-40% in Britain whereas registration rates for cataract, glaucoma, and optic atrophy have decreased (Evans and Wormald 1996).

Epidemiologic designs of population-based studies report prevalence, incidence and progression of the condition (Klein et al. 1992; Bressler et al. 1995; Mitchell et al. 1995; Vingerling et al. 1995; Cruickshanks et al. 1997; Klein et al. 1999a; Klaver et al. 2000; VanNewkirk et al. 2000; Smith et al. 2001; Klein et al. 2002; Mitchell et al. 2002a; Friedman et al. 2004a; Mukesh et al. 2004). The prevalence of a disease

describes the ratio of number of occurrences of a disease to the number of units at risk in the population for a given time period. On the other hand the incidence rate describes the number of new cases of a disease in a specific population divided by the total population and might be preferred over prevalence data because it represents the actual disease occurrence.

Klein et al. (2004) have recently published a review of the most important epidemiologic studies done in age-related macular degeneration. They summarized that late ARM is rare in persons younger than 55 years and occurs more often in persons 75 years or older. They showed that the prevalence of late ARM was less common in the black population (3.7%) than in whites (5.6%) and that there are significant variations of CNV and GA in different ethnic groups and countries. For example, prevalence and incidence of wet AMD was higher than geographic atrophy in the United States of America (USA, Beaver Dam, Wisconsin), Australia (Blue Mountains, Victoria), and in the Netherlands compared to Iceland, Norway and Greenland (Inuit) where geographic atrophy was more frequent. However, prevalence and incidence of risk factors for ARM such as drusen and RPE abnormalities were similar in whites and blacks. Nevertheless in a multiracial study performed in the USA, the rates of individual lesions suggested that non-Hispanic whites and Mexican-Americans may be protected against retinal pigment abnormalities and lesions associated with late ARM compared to non-Hispanic blacks (Klein et al. 1999b). An attempt to reduce variability was made in a three population (USA, Australia, Netherlands) based study of late ARM (Smith et al. 2001; Tomany et al. 2004b). These studies used similar grading methods and took confounders or modifiers of disease prevalence into consideration. The results

indicated no difference in the five year incidence of wet AMD or GA amongst these populations. However, it is known that the increase in incidence is not only dependent on age and race but also on certain environmental risk factors together with a genetic predisposition which might cause manifestation of ARM (Silvestri et al. 1994; Bird 2001; Bird 2003).

2.1.3 Risk factors

Several risk factors for developing early ARM or progression to late ARM have been described. Table 2.1 lists these risk factors and gives the major citations.

Table 2.1. Risk factors in ARM

risk factor	publications
increasing age	Klein et al. 1997b, Smith et al. 2001, van Leeuwen et al. 2003b
Alzheimer's disease	Blanks et al. 1991, Klaver et al. 1999, Dentchev et al. 2003
higher birth weight	Hall et al. 2002
cataract surgery	Freeman et al. 2003, Wang et al. 2003b
lower (plasma and retinal) carotenoid status	Hammond et al. 1997, Berendschot et al. 2000, Mares-Perlman et al. 2001, Gale et al. 2003, Koh et al. 2004; Richer et al. 2004
cardiovascular risk factors (systolic blood pressure, atherosclerosis, high blood cholesterol, plasma fibrinogen)	Klein et al. 1997a; Klein et al. 2003a; Klein et al. 2003b, Smith et al. 1998, Hyman et al. 2000, van Leeuwen et al. 2003c; van Leeuwen et al. 2004a, Cimbaldas et al. 2004
gender (increased in females), hormones (protective role of oestrogen)	Smith et al. 1997; Smith et al. 2001, Snow et al. 2002
medication intake (increased with b-blocker, decreased with hormone replacement therapy and tricyclic antidepressants)	van Leeuwen et al. 2004b
nutrition (role of fruit and vegetables, dietary fats)	Mares-Perlman et al. 1995, Smith et al. 2000, Cho et al. 2001; 2004, Heuberger et al. 2001, Seddon et al. 2001
ocular pigmentation (light hair, iris colour and less fundus pigment)	Weiter et al. 1985, Frank et al. 2000, Nicolas et al. 2003
race (decreased in Afro-Americans)	Gregor and Joffe 1978, Rosenfeld 1987a; 1987b, Klein et al. 1995, Schachat et al. 1995, Moeller and Mares 2003
refraction (increased for hypermetropes)	Ikram et al. 2003, Wang et al. 2004
smoking	The Eye Disease Case-Control Study Group 1992, Smith et al. 1996, Mitchell et al. 2002b, Tomany et al. 2004b
sunlight exposure	Cruickshanks et al. 1993; 2001, Darzins et al. 1997, Tomany et al. 2004a

Some of the findings have been questioned possibly because of not adjusting for all confounders or modifiers or because of insufficient statistical power. While large population based trials have included several thousands of participants, many other studies have used smaller sample sizes. Nonetheless, studies show consistent results regarding risk factors for early ARM relating to increasing age (Smith et al. 2001; van Leeuwen et al. 2003b) and smoking in developing late ARM complications such as CNV (Gass 1973; The Eye Disease Case-Control Study Group 1992; Klein et al. 1993; Smith et al. 1996; Klein et al. 1998; McCarty et al. 2001b; Mitchell et al. 2002b; The Beaver Dam Eye Study 2002; van Leeuwen et al. 2003b).

The Beaver Dam Eye Study (Klein et al. 1997b; Klein et al. 2002) has shown that age is an important factor in ARM precursors. Individuals over 75 years had a significantly higher incidence (between 4% and 26%) of either soft drusen, RPE abnormalities or late ARM signs than people between 40 and 50 years. Those with larger numbers of hard drusen (more than eight) had an increased risk of developing soft drusen and RPE abnormalities (between 5% to 12%) and there was an increased risk for eyes with soft drusen and RPE abnormalities developing late ARM (between 15% to 20%).

The Rotterdam Eye Study (van Leeuwen et al. 2003b) investigated the risk and normal course of ARM during a 6.5 year follow up and showed that the progression of ARM followed after the appearance of soft drusen gradually from a lower to higher level and accelerated for older subjects. The five year risk of developing late ARM increased with more severe stages of early ARM to 28 % in subjects aged 55 years and older. Subjects older than 80 years had increased this risk to 42 %.

Recently the Visual Impairment Project (Mukesh et al. 2004) has shown in a five year follow up study that one person in three aged 70 years or older will have ARM lesions and will progress to a more severe form after 80 years of age. The findings of soft indistinct drusen and RPE abnormalities increased the risk of developing late ARM by 9.5 times compared to those who only had drusen or RPE abnormalities. The 5-year risk for developing CNV has been described to be threefold when any of the types of drusen (hard, soft distinct or soft indistinct) were seen together with RPE abnormalities (van Leeuwen et al. 2003b).

The risk for developing CNV in the fellow eye has been estimated to be 6-18% depending on the type of late ARM (Strahlman et al. 1983; Holz et al. 1994b; Macular Photocoagulation Study Group 1997; Pauleikhoff et al. 2002; Prenner et al. 2003). A large pooled population based study demonstrated no apparent differences in risk factors for CNV compared with GA although a twofold higher incidence rate (0.4 %) for CNV compared to GA (0.2%) has been described (Klein et al. 1997b; Smith et al. 2001). Cigarette smoking, hypertension and cataract surgery have been shown to increase the risk of progression to CNV in most studies (Klein et al. 2004). Pooled findings from three continents also have demonstrated that apart from age, tobacco smoking was the only risk factor consistently associated with any form of AMD at all sites separately and in the pooled analyses (Smith et al. 2001).

The association between ARM as a risk itself for earlier mortality has been investigated in the Rotterdam Study (Borger et al. 2003). The authors reported that ARM was a predictor of shorter survival because it had risk factors that also affected mortality. However, when adjusting for these factors ARM was not associated with

mortality. A significantly higher risk for depression has been demonstrated in subjects with AMD (Casten et al. 2004). This has not only a serious impact on the lifestyle of individuals with AMD but also consequences for general health policy.

A healthy diet with reduced saturated fat, and regular consumption of fish, and rich in fruit and vegetable intake has been suggested to decrease the risk of ARM (Mitchell et al. 2003; Vaicaitiene et al. 2003). Most recently a large prospective study suggested the protective role for fruit intake on the risk of neovascular AMD but not on early ARM (Cho et al. 2004). While vegetables, Vitamin C, Vitamin E, lutein and zeaxanthin were not strongly related with the risk of ARM, it was stated that fruits (especially oranges and bananas) might include other constituents such as flavonoids, isothiocyanates, phenols, fibre, folate and potassium that play a protective role (Cho et al. 2004).

Dietary fat has long been associated with risk of atherosclerotic change by way of alteration in blood cholesterol levels (National Research Council Committee on Diet and Health Food and Nutrition Board 1989). It increases the level of circulation low-density lipoprotein (LDL) and these have been shown to increase risk for cardiovascular events. Given that ARM has been associated with cardiovascular diseases because of sharing some risk factors (Snow and Seddon 1999; van Leeuwen et al. 2003a), dietary fat intake and the risk of ARM has become a topic of several investigations. It has been suggested that dietary fat might alter the risk for ARM by enhancing the atherosclerotic process (Heuberger et al. 2001). Miceli et al. (2000) have hypothesized that a high-fat diet might interfere with lysosomal processing by influencing the rate of fatty acid oxidation.

The epidemiologic evidence regarding the relationship between dietary or serum lipid levels and age-related macular degeneration has been inconsistent especially regarding the type of fat. For example most studies have investigated total and/or animal fat intake and have demonstrated that a higher intake was associated with a higher rate of early (Mares-Perlman et al. 1995) or late ARM (Smith et al. 2000).

Omega-3-fatty acids (fish oils) play an important role in maintaining photoreceptor membrane fluid and retinal integrity (Neuringer et al. 1988). A decreased risk for late ARM has been observed in persons consuming omega 3-fatty acids in some studies (Smith et al. 2000; Cho et al. 2001; Seddon et al. 2003), whereas other studies have demonstrated no relationship (Mares-Perlman et al. 1995; Heuberger et al. 2001). However, a decreased risk in a population with a diet high in fish such as Inuits has not been proven, and the incidence of ARM in this group is paradoxically high in this population (Rosenfeld 1987a; Rosenfeld 1987b; Ostensfeld-Akerblom 1999). Other studies have shown that specific types of fat were associated with a greater AMD risk rather than the total fat intake per se (Cho et al. 2001; Seddon et al. 2001; Seddon et al. 2003). Seddon and colleagues (2001; 2003) have shown that especially vegetable fats (margarine, chocolate, peanut butter, nuts and processed baked goods such as potato chips, cookies and pies) and to a lesser extent animal fats such as saturated, mono-, poly- and trans-unsaturated fats (e.g. butter, cheese, ice cream) and linoleic fats (e.g. lamb, beef, pork) increase the risk of progression to AMD.

The inconsistent findings have been hypothesized to reflect the possibility that fat might be a marker for other conditions that influence risk for ARM (Heuberger et al. 2001). It has been demonstrated that high fat diets also tend to be lower in other

important nutrients and antioxidants (Mares-Perlman et al. 1996; VandenLangenberg et al. 1998). The subject selection also needs to be considered as, for example, one study has investigated the dietary habits of health professionals including nurses, dentists, optometrists, and physicians who tend to have higher health consciousness (Cho et al. 2001). However, the increasing prevalence of ARM in the Asian population (Maruo et al. 1991) as well as in countries of the Arctic circle (Rosenfeld 1987a) has become striking in the last two decades and suggests possible nutritional causes. Changes of life style, becoming more “western”, may have taken over these cultures. As a consequence a “faster living” and “fast food” population with similar “diseases” might be the result.

Given the great number of risk factors it is likely that almost every individual might be exposed to at least one of them during their lifespan. However, not everyone develops ARM which raises the question of whether some people have a predisposition for example genetic or immunological susceptibility.

2.1.4 Genetic influence

Although genetic research might be hindered due to the age of patients, a genetic predisposition has been suggested. Evidence for this is the similarity of ARM to some hereditary diseases and close concordance of presence of ARM between monozygotic twins and siblings (Bird 1991; Piguet et al. 1993; Heiba et al. 1994; Klein et al. 1994; Meyers 1994; Klaver et al. 1998; Gorin et al. 1999; Zack et al. 1999; Rivera et al. 2000; De Jong et al. 2001; Hammond et al. 2002; Tuo et al. 2004; Weeks et al. 2004; Zarepari et al. 2004). According to Bird (1991), Doyme (1899; 1910) was the first to suggest that there might be a genetic influence in the

pathogenesis of drusen. Hammond et al. (2002) subdivided subjects based on phenotypes in their twin study and demonstrated that large soft drusen were more heritable than smaller soft drusen. Moreover, they have shown that phenotypes with soft drusen greater than 125 μm or more than 20 hard drusen demonstrated a heritability of 45% and 81%, respectively.

Gorin et al. (1995) have identified a novel peripherin/RDS gene mutation associated with autosomal dominant retinal degeneration in patients from three different families. They found an overlap of clinical features with those of age-related maculopathy and suggested that photoreceptor-specific genes should be considered as potential factors for ARM. Additionally mutations of the ABCR gene (a photoreceptor cell-specific ATP-binding transporter gene) encoding Stargardt's disease has been found in 16% of AMD patients with the dry, geographic form (Allikmets et al. 1997; De La Paz et al. 1999; Webster et al. 2001). A correlation with the gene associated with Best's macular dystrophy has also been discussed (Petrukhin et al. 1998).

No gene has been identified that causes a significant proportion of ARM and it is likely that there is great polymorphism (Rivera et al. 2000). There might be no single genetic defect responsible for ARM and there are probably different genotypes with different pathophysiological mechanisms (Green and Enger 1993; Spaide et al. 2003a). This might imply different stages of ARM which are possibly independent of funduscopy appearance. It remains for investigation if other grading systems, for example based upon function, might provide better approach to describing ARM than does ophthalmoscopy.

2.1.5 Immunological and inflammatory factors

In a recent review Penfold and colleagues have discussed possible immunological aspects of ARM (Penfold et al. 2001). The existence of anti-retinal autoantibodies in various ocular diseases is well recognized and they have been found in sera of ARM patients (Dumonde et al. 1982; Penfold 1990; Guerne et al. 1991; Chen et al. 1993; Heckenlively et al. 1996). Penfold et al. (1990) have suggested anti-astrocyte autoantibodies to be an early feature in the pathogenesis of ARM especially when there were RPE abnormalities. They have speculated a chronic inflammatory component in ARM due to the involvement of fibroblasts and leucocytes in CNV, RPE and Bruch's membrane (Penfold et al. 1985). Additionally a higher incidence of the immunoglobulin alpha-2 globulin has been found in patients with RPE abnormalities (Penfold et al. 1990; Penfold et al. 2001). Johnson et al. (2000) have demonstrated an accumulation of IgG immunoglobulins in drusen as well as in the RPE cells overlying or directly adjacent to hard drusen.

The role of drusen as immunological markers has been reviewed extensively (Hageman et al. 2001). Earlier biochemical and histochemical studies show that drusen mainly consist of neutral lipids, phospholipids, glycolipids and glycoconjugates (Farkas et al. 1971; Killingsworth et al. 1990; Pauleikhoff et al. 1990b; Pauleikhoff et al. 1992; Holz et al. 1994a; Mullins et al. 1997; Curcio et al. 2001; Haimovici et al. 2001). Hageman et al. (2001) have demonstrated autoantibodies directly against drusen but also have shown that molecules including vitronectin, complement factors, amyloids, HLA-DR, fibrinogen, Factor X, prothrombin, apolipoprotein E and immunoglobulin lambda and kappa light chains can

be found in drusen. These molecules are synthesized in the RPE, retina and choroid and take an active part in cellular immune and/or inflammatory processes.

Many of these constituents are known as either acute phase inflammatory reactants or are known to mediate immune responses. Johnson and colleagues (1999; 2000) have suggested that primary RPE pathology stimulates localized complement activation. This in turn would lead to focal deposition of complement components and immunomodulatory molecules in the form of drusen. They have hypothesized that the disposed cellular debris within Bruch's membrane might further serve as an inflammatory stimulus. Striking similarities in deposit composition have been shown to other diseases such as glomerulonephrities (Mullins et al. 2000) and Alzheimer's disease (Dentchev et al. 2003). These findings underline the role of complement activation in the biogenesis of drusen as for example amyloid β peptide which is known to activate complement pathways, is found in drusen and is also a major component in Alzheimer's disease plaques.

Inflammatory mediators such as interleukin (IL)- 1β , tumour necrosis factor (TNF)- α and angiogenic cytokines such as vascular endothelial growth factor (VEGF) have been identified in surgically excised human CNVs (Oh et al. 1999). Histopathological sections of CNVs show a large number of inflammatory cells giving more the appearance of a wound-repair response (Spraul et al. 1999). It is tempting to hypothesise that the CNV is meant to assist repair but once established runs its own course in AMD because it lacks of inhibitory mechanisms to stop extensive scarring. It has been suggested that (IL)- 1β and (TNF)- α stimulate VEGF

production and that macrophages in CNVs are immunoreactive to both which might suggest an indirect angiogenic role of macrophages in CNVs (Kvanta 1995).

There are several similarities making an autoimmune, inflammatory response a possible candidate as the cause of ARM. Most autoimmune diseases strike women more often than men and can be more frequent in certain minority populations (Reeves 2004). Other less understood influences affecting the immune system and the course of autoimmune diseases include aging, chronic stress, hormones and pregnancy (Wilder 1995). Additionally the genes people inherit also contribute to their susceptibility for developing an autoimmune disease (Wilder 1995). In contrast to ARM autoimmune diseases tend to manifest in younger ages or can be triggered by specific factors (Selgrade et al. 1997). Assuming that ARM is based on an autoimmune process a trigger still needs to be identified. Whether this trigger is represented by the appearance of drusen or anti-retinal autoantibodies against specific retinal tissues might be the subject of future research. Especially in the latter case, tests measuring retinal function would be important, to reveal the site at which the retina is affected. This line of investigation could lead to immunosuppressive agents, such as cytotoxic drugs, antibody reagents and agents interfering with the action or expression of cytokines, being considered as future treatments of ARM, but is still hypothetical at this stage. However, most of the immunosuppressive agents used today are non-specific and have serious side effects (Frey 1999). This implies that research should focus more on the discovery of the cause of the disease rather than on the development of further agents. It might be a more promising approach to define the cellular mechanisms accounting for the cause of the early disease by for example correlating these to functional vision measures.

2.1.6 Oxidative stress

A causal relationship between oxidative stress and AMD has not been established yet but seems to be likely. Reactive oxygen intermediates (ROI) is a term used to describe free radicals, hydrogen peroxide or singlet oxygen which occur as by-products of cellular metabolism or as result of photochemical reactions (Dargel 1992). ROIs are either molecules which contain one or more unpaired radicals or are unstable and thus seek to extract electrons from other molecules or damage them. They continually leak from active sites of the enzymes involved in oxidation, a process which refers to the removal of electrons from molecules to release energy from carbohydrates, proteins or lipids. Membrane lipids, proteins and nucleic acids are especially vulnerable targets to oxidative damage. Stimuli such as irradiation, aging, inflammation pollutants (NO₂) or cigarette smoke are known to increase the production of ROI (Beatty et al. 2000b). Given that the oxygen consumption of the retina is much greater than that of any other tissue (Sickel 1972), that it is subject to high irradiation, that the photoreceptors are rich in polyunsaturated fatty acids and that the RPE as well as the neuroretina contain an abundance of photosensitisers such as lipofuscin, it is not surprising that the retina is an ideal place for the generation of ROI (Demelle 1978; Bazan 1989; Rozanowska et al. 1995; Beatty et al. 2000b). Even phagocytosis by RPE cells causes oxidative stress and results in the generation of ROIs.

It has been shown that continued oxidative injury can cause apoptosis in senescent cells (Zhang et al. 2003). Normally apoptosis is a programmed cell death and a physiologic, highly ordered cell suicide which eliminates the excessive growth of cells during embryonic development. Recent human and animal studies (Hinton et al.

1998; Dunaief et al. 2002; Gordon et al. 2002) indicate that in AMD the RPE, photoreceptors and inner nuclear layer cells die by apoptosis; the majority of dying cells are rods (Dunaief et al. 2002).

Cai et al. (2000) have used oxidative stress in an *in vitro* model to induce apoptosis in the RPE cell. They also have shown that the mitochondrial DNA was very sensitive to oxidative injury and that apoptosis is generated from accumulated mitochondrial damage during aging. Especially in non-dividing post-mitotic cells like the RPE, damage would be particularly severe. On the other hand Alge et al. (2002) have demonstrated that there are also anti-apoptotic proteins in the RPE like alpha B-crystallin which are induced by oxidative stress and are protective.

Drusen themselves might be a by-product of oxidative stress. It has been shown that soft drusen contain oxidative protein modifications including protein cross-links and thus seem to be also partly a product of oxidative injury (Crabb et al. 2002). It has been hypothesized that in ARM the physiology of Bruch's membrane might be altered; initially by accumulated cross-links with oxidatively damaged lipid and subsequent inflammatory sequela (Spaide et al. 2003a).

A possible mechanism of CNV formation due to oxidative stress could be caused by the accumulation of peroxidized lipids from the photoreceptor outer segments in Bruch's membrane. These have been identified in Bruch's membrane isolates and are mainly derived from long chain polyunsaturated fatty acids (PUFA), particularly docosahexaenoic acid and linolenic acid, which are normally found in the photoreceptor outer segments (Spaide et al. 1999). PUFAs are captured by specific

scavenger receptors (SR) such as CD-36, integrin $\alpha\beta 5$, SR-BI and SR-BII receptors on the RPE cell (Duncan et al. 2002). SR participate in PUFA internalization by RPE cells (Duncan et al. 2002) and a high concentration could lead to higher secretion of VEGF from the RPE cell (Spaide et al. 2003a). Given that peroxidized lipids preferentially accumulate in Bruch's membrane and that CNV preferentially grows there, they might be the trigger for neovascularisation (Spaide et al. 1999). Tamai et al's (2002) findings in rabbits which received injections of lipid peroxides in the subretinal space suggested that RPE cells migrate into subretinal space when exposed to oxidative stress. The histopathological correlate to focal hyperpigmentation is clumps of detached pigmented cells in the subretinal space (Bressler et al. 1994). It is known that focal hyperpigmentation are a risk factors for developing CNV (Gass 1973; Wang et al. 2003a).

However, the RPE and the neurosensory retina have their own antioxidative protective mechanism. Vitamin E is the major antioxidant of cellular membranes and is found in the RPE cell as well in the rod outer segments in high quantities (Friedrichson et al. 1995). Reduced Vitamin E levels have been shown to result in retinal degeneration and excessive lipofuscin accumulation in animals (Hayes 1978). Vitamin A (all-trans retinol) which is found in photoreceptors (Keys and Zimmerman 1999) is also involved in the repair of cells and is thought to be protected by Vitamin E (Robison et al. 1982). Carotenoids such as lutein and zeaxanthine known as macular pigment, localized in the photoreceptor axons and the processes of interneurons, act as antioxidants and are believed to limit oxidative damage by filtering the high energy blue light by about 40% (Krinsky 1979; Snodderly et al. 1984; Khachik et al. 1997). Melanin is also an important antioxidant protecting the

RPE cell (Felix et al. 1979). There are three antioxidant enzymes found in the photoreceptors and RPE which are superoxide dismutase (SOD, metalloproteins containing manganese (Mn), copper (Cu) and zinc (Zn)), catalase and glutathione peroxidase. A decrease in the RPE antioxidant capacity, especially the catalase activity, and a decreased function of antioxidant systems like vitamin E and glutathione (GTH) in the plasma have been found during aging (Vandewoude and Vandewoude 1987; Liles et al. 1991; Cai et al. 2000). Zinc is the most abundant trace element in the human eye (Karcioglu 1982) and acts as a cofactor for the antioxidant enzymes superoxide dismutase (CuZn-SOD) and catalase (Sigel 1983). It is also involved in the interconversion of all trans retinol (vitamin A) to retinal an essential part of rhodopsin synthesis (Solomons and Russell 1980). Additionally zinc induces the synthesis of metallothionein which is a scavenger of hydroxyl radicals and stabilizes membrane lipids against oxidation (Thomas et al. 1986).

Although considerable research has been carried out in this field the concept that ARM is the outcome caused by cumulative oxidative damage still remains unproven. The development of complex *in vivo* models is necessary to understand cellular mechanisms and to show the causal link between tissue damage by ROI and the onset or progression of ARM (Beatty et al. 2000b).

2.1.7 Tissues affected by ARM

Although vision loss results in ARM from photoreceptor distortion and damage, the primary site affected is still debated. It is known that the tissues involved in ARM are the choroid, Bruch's membrane, retinal pigment epithelium (RPE) and photoreceptor cells. Changes in any of these might cause reactive changes in the others as they are

metabolically interdependent (Young 1987; Bird 1991; Pauleikhoff et al. 1991; Chen et al. 1992; Curcio et al. 1996; Grunwald et al. 1998; Guymer et al. 1998; Pauleikhoff et al. 1999).

2.1.7.1 Choroid

There is evidence that choroidal hemodynamics and ischemia are responsible for ARM development. Reduced choroidal blood flow and prolonged choroidal filling in fundus fluorescein angiography (FFA) and indocyanine green angiography (ICG-A) have been associated with early and late ARM (Chen et al. 1992; Friedman et al. 1995; Zhao et al. 1995; Grunwald et al. 1998; Ciulla et al. 1999; Harris et al. 1999; Pauleikhoff et al. 1999; Ciulla et al. 2001b). It has been hypothesized that this, together with the age-related thickening of Bruch's membrane reduces the oxygen supply to the neurosensory retina. Later in the course of the disease hypoxia might then lead to angiogenesis stimulation and formation of CNV (Moore et al. 1995; Campochiaro 2000; Lip et al. 2001; Ryan et al. 2001; Witmer et al. 2003).

Hypoxia induces VEGF production in retinal cell cultures (Aiello et al. 1995) and VEGF initiates angiogenesis. Moreover recent evidence demonstrated a central role of VEGF-A in the development of CNV (Kvanta et al. 1996; Ohno-Matsui et al. 2001; Schlingemann 2004). It has been suggested that other angiogenic factors such as angiopoietins (ang-1 and ang-2) and their receptors (Tie1 and Tie2) are also involved in late ARM (Otani et al. 1999; Hangai et al. 2001). VEGF-A has been found in fibroblasts and in the RPE of surgically excised CNVs as well as in RPE cells from macula tissue of patients at high risk of developing CNV (Kliffen et al. 1997). Physiologically VEGF-A acts as a survival factor for endothelial cells and is

involved in the maintenance of function of the RPE “resting on” the choroid (Fig 2.2). This paracrine relation might be disturbed in ARM by a thickened Bruch’s membrane (Schlingemann 2004). VEGF-A is secreted on the basolateral side of the RPE cell towards the choriocapillaris. Secretion is increased by hypoxia suggesting a hypoxia-driven feedback mechanism between the RPE and the choriocapillaris (Blaauweegers et al. 1999).

Schlingemann et al. (2004) have hypothesized that aging changes in Bruch’s membrane may cause hypoxia in the outer retina from choriocapillaris atrophy. This might stimulate the RPE cells to produce more VEGF which accumulates due to the thickened Bruch’s membrane acting as a barrier. In the presence of localized defects in Bruch’s membrane (such as atrophy) this might initiate CNV.

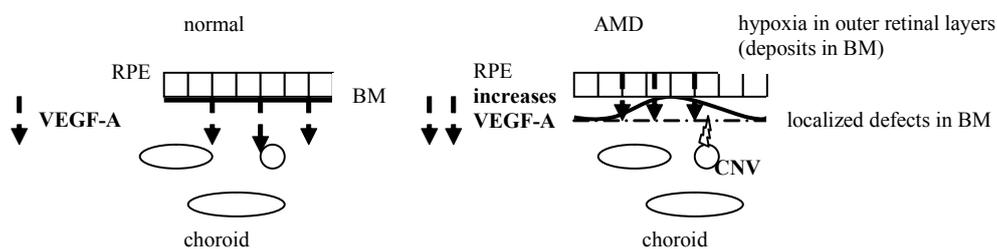


Figure 2.2 The schematic model of a possible mechanism involved in CNV formation. (BM: Bruch’s membrane)

Nevertheless there are numerous other choroidal and retinal ischemic conditions such as choroidal vascular occlusion seen in toxemia of pregnancy or malignant hypertension which are not associated with CNV (Spaide et al. 2003a). However, this might be a consequence of the younger ages of those affected by these conditions, resulting in a better regeneration function of the RPE\Bruch’s membrane complex, less accumulated aging products and thus less primary injury.

2.1.7.2 Bruch's membrane

An extensive overview of Bruch's membrane pathology and its role in aging and in the pathogenesis of ARM has been given by Guymer et al. (1998). Two major kinds of deposits depending on their ultrastructural location have been classified within Bruch's membrane during aging; basal laminar and linear deposits (Hogan and Alvarado 1967; Sarks 1976; Grindle and Marshall 1978; Newsome et al. 1987; Bressler et al. 1994; Marshall et al. 1994) (Fig. 2.3).

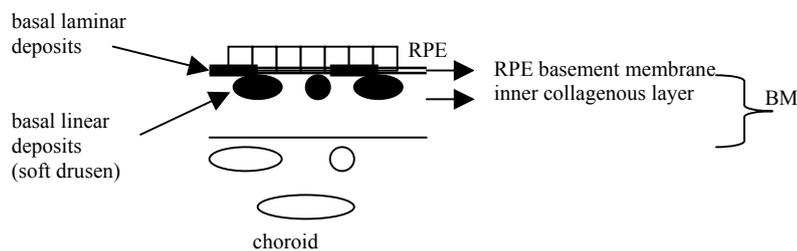


Figure 2.3: Location of basal laminar and basal linear (soft drusen) deposits ultrastructurally. While basal laminar deposits can be found between the RPE cell plasma and its basement membrane, basal linear deposits are located within the inner collagenous layer of Bruch's membrane (BM).

Basal laminar deposits are complex composites that mainly consist of fibrous collagen and coated membrane bodies and are located between the RPE cell plasma and basement membrane (van der Schaft et al. 1991; Green and Enger 1993; van der Schaft et al. 1994; Grossniklaus and Green 1998; Green 1999). This term is used to describe the widely-spaced collagen and minor deposits of material ultrastructurally and should not be confused with basal laminar drusen. Those describe the histopathological feature of diffuse drusen seen in middle aged patients with small, numerous drusen with vitelliform-like lesions (Gass et al. 1985; Kenyon et al. 1985; Bressler et al. 1994). Basal linear deposits describe a vesicular and granular electron dense, lipid rich material ultrastructurally and are located on the inner collagenous layer of Bruch's membrane. They are mainly composed of membranous debris which

can be clinically diagnosed as soft drusen (Sarks 1976; Sarks 1980; Green and Enger 1993; Sarks et al. 1994; Curcio and Millican 1999).

The origin of drusen and their molecular composition or mechanism of formation have been discussed extensively (Yanoff and Fine 1975; Sarks 1980; van der Schaft et al. 1991; Green and Enger 1993; Bressler et al. 1994; Sarks et al. 1994; van der Schaft et al. 1994). Sarks and colleagues (1976; 1980; 1988; 1994; 1999) determined that soft drusen develop from breakdown of the hyaline content of hard drusen (soft clusters) which later form localized accumulations of membranous debris within Bruch's membrane. It has been suggested that soft drusen are derived from cellular remnants of degenerated RPE cells causing an inflammatory response, and also from the choroidal vasculature and photoreceptors (Yanoff and Fine 1975; Hageman et al. 2001; Anderson et al. 2002; Crabb et al. 2002). Farkas et al. (1971) have suggested that lysosomal breakdown initiated a pathological autolysis of the RPE and that this resulted in drusen formation.

Basal laminar and basal linear deposits contribute to a thickened Bruch's membrane and to a decrease in hydraulic conductivity (Bird and Marshall 1986; Bird 1991; Moore et al. 1995; Starita et al. 1995). It has been demonstrated that the region of decreased conductivity is located at the posterior pole where there is maximal lipid accumulation (Bird 1991; Holz et al. 1994a; Starita et al. 1995). Besides inhibiting the metabolic input to the neural retina, Bruch's membrane is thought to become a hydrophobic barrier due accumulated debris blocking the normal RPE mechanism of

actively pumping fluid from the retina to the choroid. This may finally lead to the break down of the normal diffusion capacity of Bruch's membrane resulting in an RPE detachment (Fig 2.4).

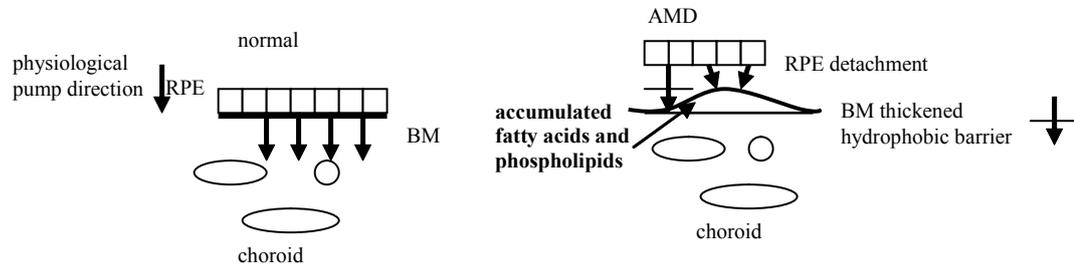


Figure 2.4. Schematic model showing RPE detachment because of BM becoming a hydrophobic barrier.

There is also evidence that advanced glycation end products (probably lipofuscin components) accumulate within Bruch's membrane. Advanced glycation end products influence growth factor production as they have been shown to elevate VEGF and pigment epithelium derived factor (PEDF) production in RPE cells *in vitro* (Handa et al. 1998; Handa et al. 1999).

Although there is no proven established pathogenesis concept, a thickened Bruch's membrane could explain the impaired psychophysical visual function findings observed in ARM. A thickened Bruch's membrane could explain slowed dynamic functions such as delayed dark adaptation (Eisner et al. 1991; Curcio et al. 2000; Phipps et al. 2003). It is known that dark adaptation represents the recovery of several aspects of the visual cycle such as rhodopsin regeneration (Leibrock et al. 1998; Lamb and Pugh 2004). Thus due to thickened Bruch's membrane, transport of Vitamin A which plays an important role in the visual cycle, from the choroid to the RPE is hindered (see also 2.1.1.7.4 and Fig 2.6). Further, reduced oxygen supply through thickened Bruch's membrane to the neurosensory retina might be another

reason for impairment of other vision functions involving, for example, the postreceptoral sites which are more vulnerable to hypoxia (Bui et al. 2003).

2.1.7.3 Retinal pigment epithelium

The RPE seems to play a major role in the development of ARM (Boulton 1991; Boulton and Dayhaw-Barker 2001). It is known for its high degradation rate of metabolic by-products of the visual cycle and the provision of protection against oxidative stress (Cai et al. 2000). RPE cells have little ability to divide and under most conditions the RPE cell persists for the life of an individual (Gao and Hollyfield 1992). The degradation of photoreceptor disc membranes within phagolysosomes and autophagic processes associated with the renewal of endogenous components, demand high lysosomal activity throughout life. It is estimated that each RPE cell will phagocytize three billion outer segment discs during the lifespan (Marshall 1987) so that eventually incompletely degraded membrane material can build up in the cytoplasm of RPE cells, and a large amount of polyunsaturated fatty acid and protein debris may be accumulated. Some of this residue is discarded through Bruch's membrane to be cleared by the choroidal circulation and part remains as lipofuscin in the lysosomes of the RPE cell.

Lipofuscin is a diverse group of brown or yellow autofluorescent molecular species which preferentially accumulates in postmitotic cells (Feeney 1978; Feeney-Burns et al. 1980; Eldred and Katz 1988; Eldred and Lasky 1993). It consists of at least of 10 different fluorophores and its autofluorescence can be measured with a scanning laser ophthalmoscope (Eldred and Lasky 1993; von Rueckmann et al. 1995). Lipofuscin is almost non existent in oxygen-free conditions (Li et al. 1999) and can

be found in the macula to a greater extent than in the periphery (Hayasaka 1989). In older people lipofuscin can occupy up to 25% of the RPE cell and can inhibit the RPE lysosomal and antioxidant activity thus leading to mitochondrial dysfunction (Schuett et al. 2000; Shamsi and Boulton 2001). One of the major lipofuscin components of the fluorophores is A2-E. A2-E consists of two compounds present in the outer retina; one is ethanolamine which is a component of the membrane lipid phosphatidylethanolamine and the other is retinaldehyde which is the oxidized form of vitamin A. A2-E has been shown to impair the lysosomal degradation functions of the RPE cells *in vitro* by elevating the intralysosomal pH (Holz et al. 1999b). While this is light independent it also has been demonstrated that lipofuscin has a light-dependent, preferential short wavelength, photoreactive function which causes oxidative damage (Boulton et al. 1993; Rozanowska et al. 1995; Schuett et al. 2000; Suter et al. 2000).

It is known that the RPE expresses a variety of growth factors which are FGF, TGF- β , IGF-I, PDGF, VEGF, TNF- α and members of the interleukin family (Campochiaro 1998). Most of their functions in the retina remain to be clarified. For example angiogenic factors such as VEGF and anti-angiogenic factors such as pigment epithelium-derived factor (PEDF) seem to be kept in relative proportion in the normal RPE cell. It is thought that the presence of deposits, particularly lipids may affect the ability of the growth factor to diffuse through Bruch's membrane (Glaser et al. 1985; Glaser 1988; Dawson et al. 1999; Spaide et al. 2003a; Schlingemann 2004). Moreover the RPE not only synthesizes growth factors but also expresses receptors for most of them. Although the exact trigger for CNV is still unknown, an imbalance between VEGF and PEDF with increased secretion of VEGF

is thought to contribute to the development of choroidal neovascularisation (Mousa et al. 1999; Miller et al. 2003; Witmer et al. 2003; Bhutto et al. 2004; Grossniklaus and Green 2004).

Fundus autofluorescence because of lipofuscin has been associated with chorioretinal neovascularisation (Delori et al. 1995; Spaide 2003) but it also occurs in eyes with geographic atrophy (Holz et al. 1999a). Holz and colleagues (1999a; 2001) showed a strong correlation between lipofuscin autofluorescence and geographic atrophy indicating that its accumulation may have an important impact on the development of dry AMD. In patients with unilateral vision loss from AMD, irregular autofluorescence in one eye was associated with geographic atrophy in the fellow eye, and predicted the development of geographic atrophy in the good eye (Holz et al. 2001). Spaide et al. (2003) demonstrated a larger degree of autofluorescence in fellow eyes of patients with CNV than in eyes of patients without history of CNV.

Although a linear relationship between age changes in the RPE and Bruch's membrane has been found there is a large between-subjects variability (Okubo et al. 1999). A change in one tissue is not necessarily associated with a change in the other. Given the various functions of the RPE it is understandable that it might reach a point where its capacity is overloaded despite sufficient Bruch's membrane capacity. This point might be genetically determined for each person depending for example on the speed of outer segment renewal, RPE degradation activity, free radical scavenging activity and RPE recycling ability. Given the RPE's crucial role in the retinoid processing cycle (vitamin A conversion to 11-cis-retinaldehyde) (Lamb and Pugh 2004) its alteration would also affect visual function substantially. This might

be independent of a thickened Bruch's membrane which is thought to delay retinoid delivery.

2.1.7.4 Photoreceptors

The anatomical macula which is about 6 mm in diameter contains two subregions with different photoreceptor populations (Osterberg 1935; Curcio et al. 1990; Gao and Hollyfield 1992). Curcio et al. (1990) demonstrated that there is a small cone dominated fovea (0.8 mm in diameter or 2.75°) and a rod-dominated parafovea (highest density between 2 to 5 mm or between about 5 to 15°). In healthy young adults the rods outnumber the cones in the macula by 9:1. Thus the macula is only cone-enriched compared to the rest of the retina (20:1) and not cone-dominated. Until recently AMD has been thought to mainly affect the cone photoreceptors. However, histological findings have indicated a preferential loss of the parafoveal rods in early ARM. Cones in the same area were misshapen but the central retinal cones looked to be the same as those in age-matched eyes with normal vision (Gao and Hollyfield 1992; Curcio et al. 1996; Curcio et al. 2000; Curcio 2001).

In their histopathological studies on healthy eye sections of different age-groups Curcio et al. (1993) showed that the spatial density of the parafoveal rods decreased by about 30% during adulthood whereas cones were spared. These findings were supported by Panda-Jonas et al. (1995) who also detected a significant rod loss during aging in healthy eyes. However, Jackson et al. (1998) tested scotopic sensitivity in older subjects and could not find an impairment in the parafoveal area. They explained this by Curcio's findings that rod outer segments "filled in" the gaps left by degenerating rods, compensating for lost rod function. The natural loss of rods

during aging has been demonstrated to be very slow (2 rods/ mm² per year) and a loss of foveal cones could be detected only in eyes older than 90 years (Feeney-Burns et al. 1990; Curcio 2001).

In another study Curcio et al. (1996) investigated 13 early ARM eyes with drusen and RPE abnormalities and eyes with CNV. They found retention of foveal cones but degeneration with expanded inner segments of the parafoveal cones in the early ARM eyes. The parafoveal cone inner segments were adjacent to each other demonstrating dramatic rod loss (especially between 0.5-1mm from the fovea) (Curcio et al. 1996; Curcio 2001) (Fig 2.5).

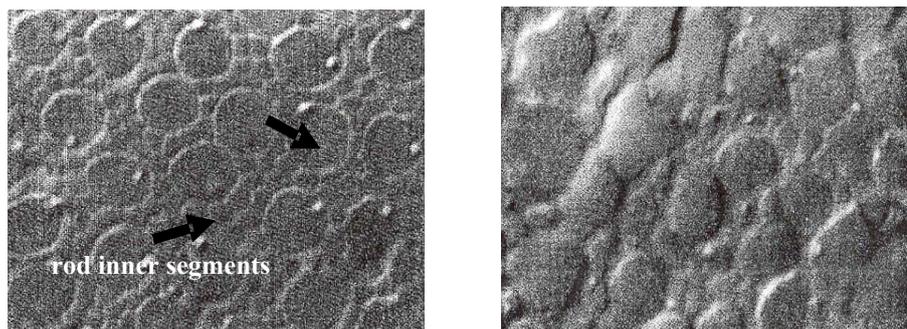


Figure 2.5. [modified from Curcio et al. (2001)] A. The normal parafovea of an older subject with regularly shaped cone inner segments and adjacent rod inner segments. B. The parafovea of a subject with early ARM shows misshapen cone inner segments and very few rod inner segments.

Although parafoveal photoreceptor loss affected cones as well, most patients showed a rod loss which exceeded cone loss by about 75% to 97% (1 mm from the fovea). Curcio et al. (1996) demonstrated that the eye with the most parafoveal rod loss was the fellow eye of an eye which had developed neovascularisation. The main photoreceptor loss was confined to a parafoveal annulus from 0.5 to 3 mm (1.5-10°) eccentricity where density was reduced to 60%-70% compared to healthy controls. In eyes with CNV the last surviving photoreceptors were largely cones adjacent to disciform scars whereas rod density decreased toward the margins of the scar.

Interestingly, the surviving photoreceptors were largely long (L-) and middle (M-) wavelength sensitive cones and these outnumbered the combined rod and short (S-) wavelength sensitive cone population in exudative ARM.

In summary Curcio and colleagues (1993; 1996) hypothesised that ARM begins in the parafovea and that the normal age-related loss of rods might be a sign of subclinical ARM. They suggested a model with the following sequence of events: “Rods die in older eyes without evidence of overt RPE disease. In some individuals, the RPE becomes dysfunctional, rod loss continues and cones begin to degenerate as well. Eventually, only degenerative cones remain. Small islands of cones may be capable of subserving vision. Ultimately, all photoreceptors may disappear if geographic atrophy or disciform degeneration develops.”

Psychophysical studies measuring rod- and cone-mediated function have confirmed that rods are first affected (Jackson et al. 1999; Jackson and Owsley 2000; Owsley et al. 2000; Owsley et al. 2001). Although photopic sensitivity was also reduced in ARM patients rod dysfunction was greater in 87% of the patients (Jackson and Owsley 2000) (see also following section 2.1.8).

Curcio et al (2000) explained preferential rod vulnerability with their “retinoid or Vitamin A deficiency hypothesis”. This model would explain slowing of the rod-mediated dark adaptation and would link photoreceptor degeneration with age-related changes in Bruch’s membrane. It is well known that vitamin A deficiency causes a slowing of dark-adaptation and that retinoids are required for photoreceptor survival (Dowling and Wald 1958; Kemp et al. 1988). Experiments with rats have

demonstrated that vitamin A deprivation leads to photoreceptor death affecting the rods first and then the cones (Carter-Dawson et al. 1979). The retinoid cycle normally comprises several sequences of events (Lamb and Pugh 2004). In brief the 11-cis retinal (cis-RAL) chromophore within the visual pigment (rhodopsin or its cone equivalent) in the photoreceptor absorbs a photon of light and is isomerized to the all-trans retinaldehyde (trans-RAL) configuration which activates the phototransduction cascade. Several forms of intermediates, still in all-trans form are generated and called metarhodopsins (identified by their absorption spectra). In the final steps all-trans retinaldehyde (trans-RAL) is reduced to all-trans retinol (trans-ROL, Vitamin A), removed from the photoreceptor outer segment and incorporated by the RPE. Within the RPE Vitamin A is converted back to its 11-cis retinal form (cis-RAL). The fundamental concept is that the delivery of 11-cis retinal to opsin in the photoreceptor outer segments is crucial for the recombination of opsin with 11-cis retinal (cis-RAL) and for the regeneration of visual pigment (Lamb and Pugh 2004) (Fig 2.6).

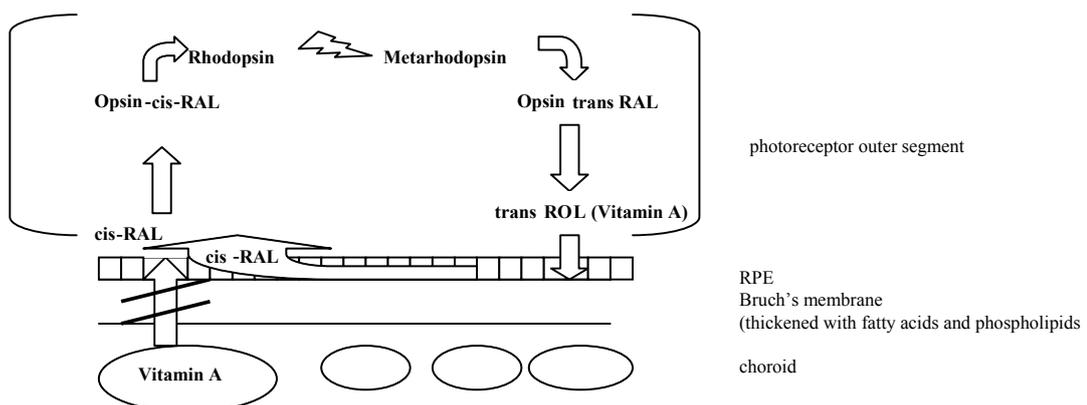


Figure 2.6. Schematic model of the reduced delivery of retinoids to the visual cycle due to thickened Bruch's membrane in ARM.

Thus, due to abnormal deposits within Bruch's membrane, dark adaptation might be delayed by reduced or slowed supply of Vitamin A (trans ROL, an important

precursor for 11-cis retinal) from the choroid. This could lead to altered precursor uptake, enzyme activity or substrate availability and localized scarcity of 11-cis-retinal. Curcio et al. (2000) further strengthened their hypothesis with the fact that there are certain late-onset diseases with typical sub-RPE deposits, such as Sorsby's fundus dystrophy or membranoproliferative glomerulonephritis type II, which also show rod dysfunction. Improvement of dark adaptation has been demonstrated in patients with Sorsby's fundus dystrophy who received Vitamin A supplements (Jacobson et al. 1995).

The most recent strong evidence of preferential rod vulnerability has been provided by Scholl et al. (2004). Scholl et al. (2004) tested photopic and scotopic sensitivity in ARM patients and performed fine matrix mapping using a modified field analyser and a modified confocal scanning laser ophthalmoscope to image fundus autofluorescence. They demonstrated reduced scotopic sensitivity in areas with increased FFA relating to early and late ARM fundus changes while photopic sensitivity was normal or mildly abnormal.

The reason why rods are affected first is not definitely known. It has been suggested that outer segment membrane components might be genetically abnormal and this together with environmental factors initiates a process of incomplete digestion in the RPE cell (Curcio et al. 1996; Gorin et al. 1999). This would be followed by an abnormal build up of lipofuscin in the RPE cell, deposition of debris within Bruch's membrane and finally the development of ARM. Additionally it has been shown that the amount of lipofuscin accumulation in the submacular RPE was proportional to the amount of overlying photoreceptor cell loss (Marshall 1987; Dorey et al. 1989).

In normal eyes the density of lipofuscin granules was maximal in the parafovea in accordance with the distribution of the rod photoreceptors, reflecting high metabolic activity (Osterberg 1935; Wing et al. 1978; Marshall 1987; Boulton and Dayhaw-Barker 2001).

There is ample evidence that rod-mediated function is impaired in early ARM (see also 2.1.8.2.2). It is surprising that no objective vision test has confirmed these findings to date. Full-field ERGs, measuring the overall rod response have not proven to be useful (Jackson et al. 2004). Of particular interest would be how histopathological findings correlate with objective function tests that measure local function. This knowledge would allow investigation of basic disease mechanisms in ARM but is beyond the scope of this thesis.

2.1.8 Diagnosis

2.1.8.1 Imaging methods

Well recognised protocols define how to assess fundus photography and use templates based on international grading systems (Klein et al. 1991; Bird et al. 1995; Age-Related Eye Disease Study Research Group 2001a) and have facilitated diagnosis of different stages of ARM. Different severity stages based upon funduscopic changes can be identified. The grading systems have been developed to give ARM studies more uniformity and make them comparable (Klein et al. 1991; Bird et al. 1995; Age-Related Eye Disease Study Research Group 2001a; Scholl et al. 2003). They all recommend the use of photographic standards of various abnormalities and a standard grid for measuring area and size of abnormalities. The grid templates are based on longstanding clinical convention of considering the

diameter of the average optic disk to be 1500 μm . The standard grid is formed by opaque lines on a transparent background and consists of three concentric circles with the 1500, 3000 and 6000 μm in diameter and is centred on the foveola. Four radial lines in oblique position directions further divide the central area into subfields. Photographic fields 30° or 35° in diameter have become the standard used in most studies with a colour stereoscopic fundus camera. Five open circles printed on clear plastic with sizes 63 μm , 175 μm , 125 μm , 250 μm and 500 μm can be used to estimate drusen size and area covered by drusen, or by increased or decreased RPE pigmentation.

Fundus fluorescein angiography (FFA) and indocyanine green angiography (ICG-A) are well known and indispensable tools in imaging and diagnosis of ARM (Shikano and Shimuzu 1968; Yannuzzi et al. 1992; Holz et al. 1997). In early ARM drusen and RPE abnormalities can be identified angiographically as either focal areas of hyper- or hypofluorescence and FAA as well as ICG-A reveal prolonged choroidal filling (Pauleikhoff et al. 1999). Wet AMD is divided into a classic CNV with a typical well demarcated netlike appearance in the early phase of the angiogram and a progressive leakage of fluorescein dye in the late phase. Classic CNV is located between the RPE and the neuroretina and thus can be better identified by FFA. In contrast occult CNV is poorly demarcated due to its location below the RPE.

Three different forms of occult CNV can be distinguished by fluorescein angiography; serous RPE detachment, fibrovascular RPE detachment and late leakage of undetermined source. These features mainly exhibit irregular stippling with ill-defined borders, early leakage and persistent staining of fluorescein in the

late angiogram. ICG-A has provided improved imaging of the choroidal circulation compared to fluorescein angiography (Yannuzzi et al. 1992). This diagnostic technique uses a water-soluble tricarboyanine dye that absorbs and emits light in the near-infrared range. The shorter wavelengths are able to penetrate pigment, blood and sero-sanguinous fluids that often obscure the image of the membrane in conventional FFA. However, both methods are invasive and can have side effects such as transitory reduction in visual acuity, altered luminance and colour sensitivity and even anaphylactic reactions (Bloome 1980; Friedman et al. 1994; Sankeralli et al. 2000).

A relatively new technique in optical imaging is the optical coherence tomography (OCT) (Hee et al. 1995). In contrast to standard ultrasound techniques which depend on reflection of high frequency sound waves, OCT uses reflection of short coherence (same frequency and phase) length laser light (usually 830 nm wavelength). This results in higher resolution images ($< 8\mu\text{m}$) compared to ultrasound images (about $150\mu\text{m}$). Although OCT cannot replace FFA in ARM it can illustrate the different features of early and late ARM very well (Hee et al. 1996). Unfortunately in late ARM OCT-images are non-specific and not very helpful in diagnosis (Thomas and Duguid 2004). In future an ultra-high-resolution OCT which is in development might give more specific results in late ARM (Drexler et al. 2001).

In addition to the acquisition of angiogram images the multiple applications of a scanning laser ophthalmoscope (SLO) have been shown repeatedly in ARM. For example SLO microperimetry allows objective focal sensitivity measures and has successfully documented sensitivity losses in late ARM (Schneider et al. 1993;

Schneider et al. 1996; Rohrschneider and Bueltmann 2001; Fujii et al. 2003). It has been proven to be useful in monitoring treatment effects after PDT (Schmidt-Erfurth 1999; Schmidt-Erfurth et al. 2004) or in documenting visual performance such as reading speed in late ARM (Ergun et al. 2003). Photopic and scotopic fine matrix mapping using a SLO to image fundus autofluorescence and a modified field analyzer have also been useful in measuring early cone and rod-mediated ARM deficits (Scholl et al. 2004). Additionally SLO combined with a tuneable infrared laser (895nm) is useful in detecting more subretinal deposits and drusen lesions than are seen in funduscopy (Elsner et al. 1995).

Other methods such as macular pigment (MP) measures quantified by heterochromatic flicker photometry (Moreland 1982; Snodderly et al. 1984; Beatty et al. 1999; Beatty et al. 2000a; Snodderly et al. 2004) or by autofluorescence using a SLO (Robson et al. 2003; Trieschmann et al. 2003) and the raman scattering spectroscopy (Lamontagne 2002; Ermakov et al. 2004) may be promising tools for detecting ARM in future. Reduced levels of MP have been associated with a higher risk of development of ARM (Gale et al. 2003). While heterochromatic flicker photometry measures the central peak absorbance of MP and is a subjective method, autofluorescence and spectroscopy measures are objective and give better information about the spatial distribution of the MP (Lamontagne 2002; Robson et al. 2003; Trieschmann et al. 2003). Latest findings of drusen and their fundus autofluorescence pattern measured with the SLO presented at the Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO) 2004, Fort Lauderdale demonstrated different patterns with increased autofluorescence indicating high risk of progression to late ARM (Einbock et al. 2004). Similarly,

macular pigment density SLO measures showed significant differences between healthy subjects and subjects with different stages of ARM (Jahn et al. 2004).

Yannuzzi et al. (2004) have given an excellent update of newer techniques for fundus imaging. They have discussed advantages and disadvantages of newer techniques such as 3-dimensional ultrasound and high-speed angiography technology. The latter has been shown to be especially useful in locating the CNV feeder vessels. The development of newer and higher resolution imaging methods also suggests the need for more accurate diagnosis of ARM. The concept of these methods is to improve tissue differentiation, even to a cellular level. With better imaging methods the use of functional tests which correlate with these images will be necessary.

2.1.8.2 Subjective macular function tests in ARM

It is important to detect ARM early and to predict late ARM before patients experience substantial vision loss and irreversible damage to the photoreceptors. Thus many studies measuring either cone- or rod-mediated functions have been performed to find tests which detect reduced retinal function sub-clinically, before significant reduction in visual acuity. Most of these studies show either impaired cone-mediated (Table 2.2) or rod-mediated function in ARM. Whether impairment is due to photoreceptor loss, misalignment of photoreceptors, postreceptoral involvement or reduced supply of metabolites important for the visual cycle, remains to be proven. For example, reduced adaptation dynamics for cones and rods with reduced post-bleaching recovery, delayed rod-cone break and slowed dark adaptation are thought to be due to slowed photopigment regeneration (“kinetic model”) (Curcio et al. 2000). This model hypothesises that due to abnormal debris within Bruch’s

membrane, transport of important metabolites of the visual cycle is hindered (Curcio et al. 2000) (see also Fig 2.5). It is thought that tests measuring steady state threshold possibly reflect different aspects of ARM-related dysfunction (Owsley et al. 2001; Phipps et al. 2003; Phipps et al. 2004).

The following sections give overviews of the most important studies investigating cone-mediated (2.1.8.2.1) or rod-mediated (2.1.8.2.2) function.

2.1.8.2.1 Subjective vision tests measuring the cone-mediated function

Table 2.2 lists the variety of cone-mediated visual functions investigated in ARM and some of the publications in this field.

Table 2.2. Cone-mediated subjective visual function tests showing impairment in early ARM

Visual function	Publications
colour discrimination (Tritan deficiency)	Collins 1986; Collins and Brown 1989b, Applegate et al. 1987, Haegerstrom-Portnoy 1988, Sunness et al. 1989a, Eisner et al. 1992
colour matching (abnormal red/green range)	Smith et al. 1988, Eisner et al. 1987a; 1987b; 1991; 1992
contrast sensitivity-spatial/temporal/colour (reduced)	Sjostrand 1979, Kleiner et al. 1988, Brown and Lovie-Kitchin 1987a, Collins and Brown 1989b, Owsley et al. 1990, Frennesson et al. 1995, Midena et al. 1997, Phipps et al. 1999; 2003, Abadi and Pantazidou 1996
visual fields (parafoveal loss)	Hart and Burde 1983; Swann and Lovie-Kitchin 1991; Cheng and Vingrys 1992, Tolentino et al. 1994, Midena et al. 1994; 1997
cone adaptation dynamics (slowed)	Brown et al. 1986b, Collins and Brown 1989a; Collins and Brown 1989b, Eisner et al. 1987a; 1987b; 1991; 1992, Cheng and Vingrys 1992, Sandberg and Gaudio 1995, Haimovici et al. 2001, Phipps et al. 2003
foveal sensitivity (loss)	Sunness et al. 1988; 1989b, Massof et al. 1989
flicker sensitivity (loss of low-and mid-temporal frequency)	Mayer et al. 1992a; Mayer et al. 1992b; Mayer et al. 1994, Phipps et al. 2004
short wavelength perimetry (decrease)	Remky et al. 2001
visual acuity distance and near (reduced)	Lovie-Kitchin 1985

Early studies in ARM have demonstrated that S-cones (Collins 1986; Applegate et al. 1987; Sunness et al. 1989a), high and low spatial frequency contrast sensitivity (Brown and Lovie-Kitchin 1987a; Kleiner et al. 1988) and paracentral visual fields (VF) are impaired in early ARM (Swann and Lovie-Kitchin 1991; Midena et al. 1994; Tolentino et al. 1994) (Table 2.2). Some of these functional tests reflect RPE abnormalities rather than drusen in the early course of the disease (Collins and Brown 1989b; Cheng and Vingrys 1992; Tolentino et al. 1994). Haegerstrom-Portnoy and Brown (1989) presented coloured spots on a coloured background to stimulate the individual L-, M- and S-cones. Stimuli were presented for 200 ms for increment threshold measures and were continuously visible for 25 Hz flicker measurements. They found a significant loss for the S-cone mechanism in the early ARM group compared to the age-matched control group. Glare recovery (Collins and Brown 1989a) or reaction time (Lovie-Kitchin and Brown 1986) have been shown to give useful information about deficits, especially in explaining the adaptational difficulties with changing light levels of early ARM subjects with “normal” visual acuity. Selective losses of flicker sensitivity in the low- and mid-temporal frequency have been demonstrated by Mayer and colleagues (1992a; 1992b; 1994) in early ARM. The stimulus used in this test was a continuously flickering, long-wavelength (660 nm) 2.8 degrees diameter circle produced from an array of 25 LEDs in an equiluminant (photopic) surround. The investigators suggested these losses were due to damage at the L and M cone photoreceptor level. Brown et al. (1986b) found elevated cone thresholds extending to peripheral areas of the retina by measuring cone adaptation over retinal eccentricities from 5° to 40° in early ARM. They used a modified dark adaptometer with a red LED stimulus (664 nm) and hypothesised that

elevation of adaptation threshold probably resulted from reduced numbers of cone photoreceptors.

While a wide range of psychophysical tests show impaired function in early ARM, it has also been shown that hallmark fundusoscopic signs such as drusen have little effect on psychophysical function. Sunness et al. (1988) measured retinal sensitivity over drusen and non drusen areas with a fundus camera stimulator. They could not find a significant difference in sensitivity between areas with hard or soft drusen or more progressed ARM in the fellow eye. Tolentino et al. (1994) showed that the number of central visual field defects increased with increasing RPE atrophy but not with drusen. Some investigators found reduced threshold sensitivities or reduced colour contrast sensitivity over drusen in early ARM (Midena et al. 1994; Frennesson et al. 1995). However, although elevations for the protan, deutan and tritan axes were shown for subjects with eyes at higher risk (with late ARM in the fellow eye), patients with more advanced ARM such as disciform scarring in the fellow eye did not show higher thresholds. Short wavelength automated perimetry has been demonstrated to be affected in eyes with drusen in which the fellow eye had late ARM (Remky et al. 2001). In contrast, short wavelength perimetry results were less affected in eyes with RPE abnormalities and focal hyperpigmentation and sensitivities were not different to that of control eyes. Most recently Phipps et al. (2004) demonstrated that static perimetry was less significantly impaired than flicker perimetry in early ARM subjects compared to a control group. The authors proposed the “metabolic challenge model” suggesting that flicker tests require higher metabolism. Thus a system that operates at its limits such as the Bruch’s

membrane/RPE /photoreceptors complex in early ARM is more challenged by flicker stimuli than by steady state stimuli.

A few investigators have shown that some tests are predictive of progression of ARM (Sunness et al. 1989b; Eisner et al. 1992). Sunness et al. (1989b) investigated photopic dark adapted sensitivity for a 1.8° red 500-msec stimulus centred at the fovea and demonstrated that the sensitivity loss at baseline predicted the progression in ARM three years later. Eisner et al. (1992) showed that eyes with slow dark adaptation, impaired colour matching and reduced S-cone mediated sensitivity were likely to develop CNV. The investigators hypothesized that a combination of dark adaptation and colour matching was highly predictive but each test by itself did not appear to be an effective risk indicator.

Several studies used a battery of psychophysical vision tests in ARM. Collins et al. (1989b) tested contrast threshold, glare recovery time, as well as visual acuity and colour vision. They showed a significant correlation between prolonged recovery time and the other function tests; prolonged recovery time was significantly associated with RPE abnormalities. Eisner et al. (1992) found reduced S-cone sensitivities, photopic dark adaptation, absolute threshold and colour vision in the eye with good vision (20/25 or better) whose fellow eye had suffered CNV. They showed that recovery of dark adaptation was slowest in eyes with the most drusen and/or atrophic changes. Cheng and Vingrys (1992) detected compromised Amsler grid, central visual fields, colour vision and photostress recovery time in their early ARM subjects. They also demonstrated a significant correlation between increased RPE abnormalities and prolonged photostress recovery time. However, the presence

of confluent drusen correlated well with losses in colour saturation but not with photostress recovery. The investigators suggested that different pathological processes might be responsible for the different functional losses. In a large study Sandberg and Gaudio (1995) measured glare recovery in 133 fellow eyes of subjects with unilateral CNV. Sixty-two percent of their patients with visual acuities of 6/18 or better had prolonged recovery times. They found good correlations between visual acuity, funduscopy changes, age and recovery time. Similar to the results of Collins and Brown (1989b) and Chen and Vingrys (1992), recovery time increased with foveal RPE atrophy. Midea et al. (1997) tested glare recovery function, central visual fields, spatiotemporal contrast sensitivity, and colour vision and found impairment in all tests except in colour vision. Phipps et al. (2003) measured spatial contrast sensitivity, temporal contrast sensitivity, colour threshold and adaptation dynamics in their early ARMs. Although steady state aspects of cone function were affected kinetic aspects showed greater deficits. The authors considered their results more likely to reflect receptor change based upon Curcio's vitamin A deficiency hypothesis (Curcio 2001). However they did not exclude a postreceptor site.

2.1.8.2.2 Subjective vision tests measuring the rod-mediated function

Dark adaptation has been shown to be affected in ARM (Campbell and Ritter 1969; Brown and Kitchin 1983; 1986a). Brown and colleagues (1986a; 1986b) were the first to measure both cone and rod recovery dynamics to red (635 nm) and green (565 nm) stimuli at different eccentricities (0, 5, 10, 25, 15°) in ARM subjects. Although there was a sensitivity loss for both cone and rod functions, they also showed that time constants for rod recovery were significantly longer for the ARM than for the

control subjects, whereas cone recovery was similar for both groups. The authors suggested that this was due to altered rod photochemistry.

Steinmetz et al. (1993) performed scotopic static threshold perimetry and dark adaptometry in subjects with early ARM. They found that scotopic thresholds, rod-cone breaks and time course of dark adaptation were impaired but varied from one subject to another. Some subjects had normal dark adapted static perimetry but abnormal adaptation kinetics and others had normal rod adaptation despite elevated final thresholds at some locations. The authors explained these differences by suggesting that progression of disease might not be uniform with rod function being affected differently in each individual. They found no correlation between quantity of drusen and severity of the functional deficit.

A more recent study by Owsley et al. (2000) examined dark- and light-adapted sensitivity at 51 different loci with static threshold perimetry using a modified field analyzer. They demonstrated either abnormal dark- or light-adapted sensitivity or both, but more subjects had impaired rod than cone dysfunction. Owsley et al. (2000) also found no correlation between the amount of drusen and sensitivities measured foveally or parafoveally, under dark-or light-adapted conditions. They concluded that their test measured different expressions of the ARM disease process.

In a following study Owsley et al. (2001) assessed kinetic rod adaptometry at 12° together with static scotopic and photopic sensitivity and contrast sensitivity in twenty early ARM subjects. While no significant differences were found between the ARM and control subjects in contrast sensitivities and visual acuities, there were on

average 0.2 and 0.4 log units lower photopic and scotopic sensitivity respectively, for the ARM subjects compared with the control group. For the dark adaptation measures the results were fitted with Lamb's model (Lamb 1981) using nonlinear regression. Besides the rod-cone break, emphasis was put on the two later components of the dark adaptation curve (rod sensitivity recovery and time to baseline) as they are thought to reflect the rate of rhodopsin regeneration. Owsley et al. (2001) found a delayed rod-cone break and slower sensitivity recovery for the ARM group compared to the control group but the last component (time to baseline) of dark adaptation was comparable with the control group. They explained delayed dark adaptometry kinetics with Curcio's retinoid deficiency model (Curcio et al. 2000) (see also Fig 2.5 and 2.1.7.4). Haimovici et al. (2002) tested dark adaptation in early ARM eyes whose fellow eyes had different stages of early to late ARM. Again, kinetic rod adaptation was more affected than cone adaptation. Rod adaptation was most abnormal in the group with the RPE detachment or RPE tear followed by the group with CNV in the fellow eye but was least affected in eyes with bilateral drusen.

In conclusion not all studies agree in their outcomes and some tests are more sensitive in some studies than in others. These discrepancies are probably due to different stages of ARM, ages of subjects, or fundus grading systems and functional qualities of photoreceptors measured with different test stimuli or even due to the subjective nature of tests. Most of the recent studies explain their impaired results using Curcio's model. However, delayed photoreceptor dynamics might also reflect a more progressed disease where there is already a great amount of abnormal deposits. Given that drusen (basal linear deposits) are thought to represent most of this

abnormal material and that there are poor correlations with dynamic function tests (Collins and Brown 1989b; Cheng and Vingrys 1992; Steinmetz et al. 1993), other causes possibly reflecting earlier deficits should be considered. For example, chronic hypoxia could contribute to poorer receptor/postreceptor performance (Bui et al. 2003) or a misalignment and distortion of the photoreceptors themselves early in the disease could cause abnormal synaptic transmission. Thus an objective, electrophysiological approach focusing on the neuronal involvement might be another way to gain a better understanding of the pathogenesis of ARM.

2.1.8.2.3 Electrophysiological assessment of ARM

Given that ARM is thought to be a disease localized to the central retina one might not expect measurable changes in full-field electroretinography. Indeed, there are contradictory findings of studies using full-field electroretinogram (ERG) and electrooculogram (EOG), some showing no change (Marcus et al. 1983; Sunness et al. 1985; Holopigian et al. 1997b) and others impairment in ARM (Henkes 1954; Niemeyer 1969; Merin and Auerbach 1970; Wu 1991; Walter et al. 1999). Sunness et al. (1985) observed normal ERG and EOG values regardless of the severity of macular degeneration. Conversely Walter et al. (1999) found significant changes compared to an age-matched control group in full-field ERGs and EOGs. They have correlated electrophysiological findings with funduscopy findings in early and late ARM. A wide involvement of the outer retina was reflected in the full-field ERGs which showed reduced scotopic and photopic a- and b-waves as well as reduced oscillatory potentials. Similarly, EOG parameters were significantly lower in the ARM group compared to the control group indicating a generalized deficit at the RPE/choriocapillaris level. However, visual acuity did not correlate with

electrophysiological data but poor EOG values were associated with eyes with more advanced ARM. The fact that they also found significant differences for the full-field scotopic ERG measures agrees with other studies (Wu 1991; Holopigian et al. 1997b). Holopigian et al. (1997b) found abnormal rod system absolute thresholds, cone-rod break times, and rod-dominated full-field ERGs in some ARM patients whereas cone measures and the EOG were within the normal range. In contrast Jackson et al. (2004) demonstrated that the full-field rod-mediated ERG was not affected in a healthy younger and older group as well as in early and late ARM subjects. They suggested that testing rod-mediated function with the mfERG might detect the parafoveal rod abnormalities seen in ARM better.

Focal assessment of macular function, and thus a better mapping of localized diseases was introduced in early studies (Arden and Banks 1966; Aiba et al. 1967; Biersdorf and Diller 1969; Sandberg and Ariel 1977; Jones et al. 1986). While difficulties in recording due to a small local stimulus and stray light from the non-stimulated area have been reported, improved techniques have been developed (Miyake et al. 1981; Seiple et al. 1986a; Seiple et al. 1986b; Yamamoto et al. 1995; Falsini et al. 2003). Sandberg et al. (1993) found a delayed latency in the foveal cone-mediated ERG in early ARMs at risk (those with fellow eye having developed CNV) compared to the control group. They demonstrated amplitudes within the normal range and suggested that delayed latencies reflected abnormal function. Remulla et al. (1995) performed foveal ERGs in fellow eyes of subjects with unilateral CNVs and found prolonged latencies to be correlated with prolonged choroidal filling on FFA and with drusen extent. They suggested that the prolonged foveal ERG latency reflected outer retinal ischaemia. Falsini et al. (2003) recorded

macular (18°) focal ERG in 19 early ARM subjects and 11 age-matched control subjects. They evaluated cone-mediated flicker sensitivity with the focal ERG based upon Mayer et al.' (1994) findings of affected low and mid temporal frequencies associated with fundus features in early ARM. They found abnormalities in response gain and phase but normal thresholds in less advanced ARMs, whereas more advanced ARMs showed additionally increased thresholds.

Sunness and Massof (1986) developed a focal EOG and found that it did not separate the ARM subjects from a control group, nor did it discriminate between the subgroups of ARM. They concluded that the focal EOG, like the full-field EOG was not a sensitive test for ARM.

To date no study has investigated the local rod-mediated responses in early ARM. The mfERG and its use in ARM are reviewed in sections 2.2 and 2.3.

2.1.9 Treatment of ARM

Current recommendations for the treatment of early and late ARM include supplementation of vitamins and antioxidants (Age-Related Eye Disease Study Research Group 2001c), conventional (argon/ krypton) laser photocoagulation (Macular Photocoagulation Study Group 1982; Macular Photocoagulation Study Group 1986; Macular Photocoagulation Study Group 1990a) or photodynamic therapy (PDT) (Treatment of Age-Related Macular Degeneration With Photodynamic Therapy (TAP) Group 1999; Bressler and Treatment of Age-Related Macular Degeneration With Photodynamic Therapy (TAP) Study Group 2001; Blumenkranz et al. 2002) which are discussed in the following two sections (2.1.9.1

and 2.1.9.2). There are several other treatments for early and late ARM which have not been established but have been successfully applied in some trials such as supplements other than those used in the AREDS prescription (Bartlett and Eperjesi 2003) or transpupillary thermotherapy (TTT) (Reichel et al. 1999), surgical removal of CNV (Lambert et al. 1992) or intravitreal triamcinolone acetonid with or without PDT (Penfold et al. 1995; Penfold 2002; Spaide et al. 2003b) which are also discussed in the last section (2.1.9.3). In addition currently ongoing studies utilizing antiangiogenic drugs are discussed in detail (Eyeteck Study 2002; Eyeteck Study 2003; The Anecortave Acetate Clinical Study Group 2003) (2.1.9.3).

2.1.9.1 Treatment of early ARM

The AREDS study (Age-Related Eye Disease Study Research Group 2001c) was initiated to investigate the effect of a combination high dose therapy (about 5 to 15 times the recommended dietary allowance) of the antioxidants vitamins C and E, zinc and betacarotene on the risk and progression of ARM over an average of 6.3 years. A total of 3609 participants were included in the analysis. This study showed that ARM subjects with extensive intermediate size drusen, at least one large druse, non-central geographic atrophy in one or both eyes, late ARM or vision loss due to AMD in the fellow eye and without contraindications such as smoking, should consider taking a supplement of antioxidants plus zinc. A criticism of the study was that many subjects chose to take additional vitamins containing at least one study ingredient (67%, out of these 30% had been taken vitamins for more than 5 years) (Abramson and Abramson 2002). Additionally carcinogenic effects of betacarotene on smokers have to be considered (The Alpha-Tocopherol Beta Carotene Cancer Prevention Study Group 1994) and patients have to be monitored carefully for toxicity and possible

adverse effects of the high dosages of ingredients. For example vitamin C can cause kidney stones, vitamin E can interfere with the action of oral anticoagulants or can decrease blood sugar levels, and zinc can cause anaemia or copper deficiency. It has been demonstrated that the potential positive public health impact in the United States would be considerable over the next 5 years if people at high risk for advanced AMD received supplements such as those suggested by the AREDS group (Bressler et al. 2003). In a recent study, Jampol (2003) strongly recommended the vitamin therapy but cautioned that dosages suggested by the AREDS group should be prescribed. Hopley et al. (2004b) most recently showed that screening for ARM and prophylactic treatment with zinc and antioxidants would be highly cost effective.

2.1.9.2 Treatment of late ARM

There is no medical or surgical treatment for dry AMD. Approximately 10-20% of patients present with the wet AMD, and the majority (80%) present with angiographically occult CNV (Bressler et al. 1987; Freund et al. 1993). The effect of treatment depends on the size of the CNV and presenting visual acuity, but is less beneficial in those with occult CNV (Verteporfin In Photodynamic Therapy (VIP) Study Group 2001b). Conventional treatment of CNV in AMD of extrafoveal (>200 μ m from the avascular zone) or juxtafoveolar (distance between 1-200 μ m from the avascular zone) lesions involves short-pulse thermal laser (argon or krypton) photocoagulation of the CNV which results in immediate chorioretinal damage and permanent photoreceptor loss (Macular Photocoagulation Study Group 1982; Macular Photocoagulation Study Group 1986; Macular Photocoagulation Study Group 1990a). This treatment is suitable for only a small number of subjects (about 15%) in whom the CNV lesion has FA patterns of classic CNV and is of small size

with well defined boundaries (Macular Photocoagulation Study Group 1991b; Freund et al. 1993; Moisseiev et al. 1995). Moreover, following laser photocoagulation there is a 50% recurrence rate of the CNV within a 3 year period (Macular Photocoagulation Study Group 1990b; Macular Photocoagulation Study Group 1991a; Macular Photocoagulation Study Group 1994b). Untreated subjects with the exudative form of AMD can be expected to lose vision within 2-3 years (Bressler et al. 1982; Soubrane et al. 1990) whereas subjects treated with juxtafoveal CNVs have been shown to have better visual acuity outcome in comparison with untreated subjects at the 5-year examination (Macular Photocoagulation Study Group 1990a; Macular Photocoagulation Study Group 1994a). Of the treated eyes 13% had better than 6/12 visual acuity compared to 5% of the untreated eyes. Similarly 25% (treated eyes) versus 40% (untreated eyes) had visual acuity of 6/60 or worse. Laser treatment of subfoveal classic CNVs has been demonstrated to reduce the long term risk (up to four years) of severe loss of visual acuity and contrast thresholds compared to untreated eyes but is accompanied with immediate central vision loss (Macular Photocoagulation Study Group 1993). Laser treatment of eyes with purely occult CNVs is of no benefit compared to untreated eyes and is not recommended (Soubrane et al. 1990).

Photodynamic therapy (PDT) is more selective in terms of the retinal layers treated and therefore is thought to cause less unnecessary damage to the cones and rods (Schmidt-Erfurth and Hasan 2000; Schmidt-Erfurth et al. 2002a). PDT is based upon a temporary or permanent occlusion of the CNV mediated by reactive excited-state oxygen species (e.g. singlet oxygen) with subsequent platelet activation and thrombosis (Schmidt-Erfurth et al. 1994; Schmidt-Erfurth and Hasan 2000; Ghazi et

al. 2001). Randomized prospective studies have shown that PDT reduced the risk of moderate and severe vision loss over a 3 year period (Treatment of Age-Related Macular Degeneration With Photodynamic Therapy (TAP) Group 1999; Treatment of Age-Related Macular Degeneration With Photodynamic Therapy (TAP) Study Group 2001; Verteporfin In Photodynamic Therapy (VIP) Study Group 2001b; Verteporfin In Photodynamic Therapy (VIP) Study Group 2001a; Treatment of Age-Related Macular Degeneration With Photodynamic Therapy (TAP) Study Group 2002). The TAP investigation demonstrated that verteporfin reduced the risk of three lines of vision loss at both 12 and 24 months compared with placebo. Subgroup analysis of the benefit was limited to eyes with predominantly classic (>50% by FFA) lesions. A beneficial functional outcome has been shown for contrast sensitivity, fixation stability and visual fields (Elsner and Schmidt-Erfurth 2002; Rubin et al. 2002; Schmidt-Erfurth et al. 2004). For minimally classic lesions (<50% but >0% of the area of the entire lesion) at baseline no statistically significant differences in visual acuity loss have been found compared to a placebo group. However, a recent study has demonstrated that 40% of minimally classic lesions have converted to predominantly classic CNVs and most of them within three months (Bressler et al. 2004). It has been suggested that subjects with minimally classic lesions should be closely monitored so that conversion can be promptly identified and PDT considered.

The VIP (Verteporfin in Photodynamic Therapy Study Group) trial was partly designed to investigate occult CNV with no evidence of classic CNV and with visual acuity better than an approximate Snellen equivalent of 20/40. The study demonstrated a beneficial outcome with respect to visual acuity, contrast sensitivity

and membrane growth in subjects with occult CNV with either lower levels of visual acuity (equal to 20/50 or worse) or smaller lesions (< four disk areas) after two years (Verteporfin In Photodynamic Therapy (VIP) Study Group 2001b). Thus the VIP study recommended considering PDT in patients with occult CNV with either a small lesion regardless of initial visual acuity, or lower visual acuity regardless of initial lesion size.

Usually a number of PDT treatments are required, with the TAP and VIP studies reporting 3.4 and 2.2 treatments in the first and second year, respectively. Histopathological studies in human eyes do not indicate that one PDT treatment damages the neurosensory retina (Schloetzer-Schrehardt et al. 2002; Schmidt-Erfurth et al. 2002a). In contrast in animal experiments damage to neurosensory retina in the treated area and collateral damage to the healthy choroid and neurosensory retina was evident (Zacks et al. 2002).

To give a better understanding of the underlying pathomechanism of PDT and the vascular changes of CNV components, vascular events have been investigated in more detail with ICG-A (Michels et al. 2002; Schmidt-Erfurth et al. 2002b; Michels and Schmidt-Erfurth 2003; Schmidt-Erfurth and Michels 2003). Vessel closure does not happen immediately after PDT but is achieved after one day. Based on early angiography assessment, PDT (one day after) results in dramatic changes in choroidal exudation with massive leakage originating from the surrounding, previously unaffected choroid. Similar effects were evident with ocular coherence tomography (Rogers et al. 2002). Most of these acute responses with massive

subretinal and subpigment epithelial edema had ceased after one month (Schmidt-Erfurth 2001; Rogers et al. 2002; Michels and Schmidt-Erfurth 2003).

One hypothesis explains these immediate inflammatory responses as damage to the vascular endothelial cells and pericytes and/or the RPE cell induced by photochemical reactions. ICG-A still reveals an apparent but substantially smaller membrane one week after treatment. Four weeks after PDT 50% regrowth (recurrences from a feeder vessel) or recanalisation (persistence) is evident (Schmidt-Erfurth and Michels 2003). ICG-A also detects hypoperfusion of the surrounding healthy retina which is fully developed after one week and still not recovered to many months in some subjects (Schmidt-Erfurth and Michels 2003).

It is still not clear whether regrowth or recanalisation is the reason for subsequent enlargement of the CNV after PDT. Schmidt-Erfurth et al. (2002b) hypothesized that chronic recurrence might be closely related to choroidal hypofluorescence. They showed that the size of the relative scotoma in microperimetry after PDT reflected PDT-induced choriocapillary atrophy (Schmidt-Erfurth et al. 2004). Schmidt-Erfurth et al. (2003) suggested that chronic malperfusion and hypoxia might induce VEGF expression. They speculated that continuous recurrence might be a consequence of VEGF (VEGFR-3) stimulation reinforced by the therapeutic intervention itself. Schmidt-Erfurth et al. (2002b) further investigated the photodynamic effects on CNV after multiple treatments and found that hypofluorescence decreased in size and intensity over time in the single treatment group. The group which had multiple PDT treatments showed CNV enlargement up to 25% compared to the single treatment

group. Although hypofluorescence decreased after multiple treatments, it did not show as much resolution as the found in the group which had only one treatment.

Currently a number of study groups are evaluating different PDT protocols by expanding the use of PDT and by investigating the current PDT scheme (Kaiser and Verteporfin In Occult (VIO) Study Group 2004; Rosenfeld and Verteporfin in minimally (VIM) Study Group 2004; Singerman and Verteporfin with Altered (delayed) Light In Occult (VALIO) Study Group 2004; Stur and Verteporfin Early Retreatment (VER) Study Group 2004). One interesting result was given by the VER who investigated shorter retreatment intervals in their late ARM subjects and showed no benefit compared to the conventional three months retreatment scheme. This might be explained by the Schmidt-Erfurth et al. (2002b) hypothesis that the PDT effect is less intense when choosing shorter retreatment intervals. They suggested that there might be less sensitizer and less oxygen available to finally target the new vessel formation when a following PDT is applied to an area with persistent vascular occlusion. However, optimal treatment protocols in terms of duration of treatment, number of retreatments, interval between retreatments as well as dosages and mechanism need further investigation.

2.1.9.3 Treatments under investigation

2.1.9.3.1 Zinc, carotenoid and antioxidant combination therapies

Although a number of clinical trials have shown some benefit of antioxidant and vitamin supplementations there is no established therapy for early ARM apart from the AREDS recommendations. Bartlett and Eperjesi (Bartlett and Eperjesi 2003) and Beatty and co-workers (2000b) have extensively reviewed the most important

clinical trials including studies utilizing zinc (zinc in macular degeneration and zinc in the second eye in AMD trial) or vitamin E (vitamin E, cataract and AMD trial VECAT (Taylor et al. 2002)) alone or in combination therapies with vitamin E, C, beta-carotene and buphenine (Visaline trial) or alpha-tocopherol and beta-carotene ATBC trial (Teikari et al. 1998). Newsome et al. (1988) investigated the effects of oral zinc supplementation on visual acuity in 151 subjects with drusen or AMD. Although some eyes in the zinc-treated group lost vision, this group had significantly less vision loss than the placebo group after a follow-up of 12 to 24 months. On the other hand Stur et al. (1996) investigated the effect of oral zinc in 112 early ARM at risk subjects (the fellow eye had CNV) over a two year period and could not show any beneficial outcome for visual acuity, contrast, colour discrimination or retinal grating acuity. The LAST study (the Lutein Antioxidant Supplementation Trial) investigated a lutein antioxidant combination (not specified) therapy to investigate its protective ability in ARM (Richer et al. 2004). They investigated 90 eyes over a period time of two years and found visual function improvement with lutein alone or lutein together with other nutrients.

The Blue Mountains Eye study (Flood et al. 2002) has investigated intake of the antioxidants alpha- and betacarotene, beta-cryptoxanthin, lutein, zeaxanthin, lycopene, retinol, vitamins A and C and zinc in early ARM based on a food frequency questionnaire and electronically calculated nutrient intakes. They found no significant association between the normal intake of any antioxidant and the 5 years incidence of early ARM and suggested much higher than usual intakes were necessary for any protection to become manifest.

The Beaver Dam Eye Study did not find any significant correlation between higher intake of carotenoids and decreased risk for developing ARM (Mares-Perlman et al. 1996; VandenLangenberg et al. 1998). The EDCC (Eye Disease Case Control Study) study showed that a high intake of carotenoids protected against late ARM (Seddon et al. 1994). The risk was especially low in subjects who frequently ate spinach and collard greens. However, Seddon et al. (Seddon et al. 1994) also showed that other nutrients applied in this study such as vitamins A, E and C were not appreciably related to ARM or were associated with a significantly reduced risk for ARM.

The macular pigments lutein and zeaxanthin are reduced in smokers (Hammond et al. 1996), a group at higher risk for developing late ARM. The NHANES (National Health and Nutrition Examination Survey) (Mares-Perlman et al. 2001) demonstrated significant inverse associations between the consumption of lutein and zeaxanthin and risk of RPE abnormalities or late ARM. However, this association was found only in younger people (between 40-59 years). For the whole population studied there was no evidence that a higher intake of carotenoids was protective against early or late ARM. Gale et al. (2003) examined zeaxanthin and lutein plasma concentrations separately, whereas most of the other studies had investigated the combination of zeaxanthin and lutein (Seddon et al. 1994; Mares-Perlman et al. 2001). Gale et al. (2003) showed that people with the lowest plasma concentration of zeaxanthin had a two-fold increase in risk of ARM compared with those with the highest concentrations. However, lower levels of lutein alone or the combination of lutein and zeaxanthin did not change the risk significantly. The authors concluded that previous investigations of combination carotenoid therapies may have obscured evidence of zeaxanthins' protective role.

The findings of many of these studies investigating macular pigment concentration need to be interpreted cautiously as carotenoid levels were measured as plasma concentrations, rather than actual density at the macula. An individual absorptive and digestive characteristics as well as the lack of sufficient data regarding the carotenoid content of foods need to be considered.

Supplemental lutein and zeaxanthin have been shown to increase the amount and density of macular pigment (Hammond et al. 1997; Landrum et al. 1997; Berendschot et al. 2000) and reduce photoreceptor death in animals (Thomson et al. 2002). In a recent study, Koh et al. (2004) measured both macular lutein density (by flicker photometry), and plasma lutein concentration in seven early ARM and six control subjects. Although macular lutein density increased with plasma lutein concentration this effect was similar for both groups.

2.1.9.3.2 Statins

Associations between risk factors (hypertension, smoking, high plasma cholesterol) for ARM and cardiovascular diseases (The Eye Disease Case-Control Study Group 1992; Klein et al. 1998; Evans 2000; Klein et al. 2003b) have led to the hypothesis that they share common pathomechanisms. Statins are thought to cause regression of pre-existing atherosclerotic disease, exert direct anti-atherosclerotic effects (Vaughan et al. 1996; Blumenthal 2000) and are lipid-lowering by inhibiting the HMG-CoA (3-hydroxy-3-methylgluaryl coenzyme A). HMG-CoA is a key enzyme for cholesterol biosynthesis and lowers LDL (low density lipoprotein) cholesterol. It is known that cholesterol accumulate in the aging Bruch's membrane (Pauleikhoff et al. 1990b; Holz et al. 1994a; Curcio et al. 2001; Haimovici et al. 2001). Significant

benefits of statins on early ARM progression and late ARM have been shown in studies with small sample sizes (Hall et al. 2001; McCarty et al. 2001a) but larger population based studies have not confirm these results (Klein et al. 2003c; van Leeuwen et al. 2003c). In the most recent study Wilson et al. (2004) retrospectively reviewed the fundus photography records of more than 100 subjects with CNV or dry AMD. They showed that subjects with CNVs and dry AMDs were significantly less likely to have a prescription for statins or aspirin than a control group. In this study subjects with CNVs were more likely to have higher triglyceride levels, lower HDL (high density lipoprotein) cholesterol and were more likely to be smokers. However, the investigators could not demonstrate a significant association between serum total cholesterol or LDL cholesterol and CNV. Thus the use of statins in the treatment of ARM requires further investigation.

2.1.9.3.3 Transpupillary thermotherapy (TTT)

Transpupillary thermotherapy (TTT) with a diode laser causing hypothermia has been reported to be useful for occult CNVs (Reichel et al. 1999), a subgroup for which PDT treatment has not yet been fully established (Verteporfin In Photodynamic Therapy (VIP) Study Group 2001b), in a small sample size of 16 eyes of 15 subjects. Based upon this pilot study, TTT has become an alternative treatment for occult CNVs. TTT was originally used for the treatment of choroidal malignant melanoma, inducing tumour necrosis mainly by a direct cytotoxic effect and a thrombosis of the tumour vessels (Oosterhuis et al. 1995; Journee-de Korver et al. 1997).

Like PDT, TTT induces damage to the neurosensory retina and the RPE. The magnitude of the damage has been shown to depend on dosage and fundus pigmentation, so the optimal TTT treatment protocol is still under investigation. Under- or overtreatment effects arise depending on the level of ocular pigmentation (Salinas-Alaman et al. 2003). Ocular hypoperfusion after TTT has been demonstrated to occur suggesting ischemic insult after treatment (Ciulla et al. 2001a; Thomas 2001; Connolly et al. 2003; Salinas-Alaman et al. 2003). A recent study by Algvere et al. (2003) showed that 59.3% of eyes with predominantly occult CNV lost fewer than 15 letters of visual acuity after 12 months. When minimal classic lesions larger than 3 mm were excluded, 62.5% of eyes lost fewer than 15 letters of visual acuity after 12 months. However, the laser spot size (of 3 mm) did not entirely cover the membrane and might have contributed to a poorer outcome in patients with larger neovascular membranes (Ciulla 2003). A controlled double-blind clinical trial (TTT4CNV) which investigates the effectiveness of TTT is currently under way and study design and patient baseline characteristics as well as baseline clinical characteristics were presented at the ARVO 2004 meeting (Elledge et al. 2004; Reichel et al. 2004). This study includes 289 subjects, 80% of whom had purely occult CNV with moderately impaired visual acuity (mean of 20/80). Follow-up is planned over a 24 month period. Preliminary medium term results of combined TTT and triamcinolone trials have been presented demonstrating a beneficial effect (Jablon et al. 2004; Newsom et al. 2004). Additionally animal models using subthreshold power setting (80 mW compared to conventional power settings usually >300 mW) demonstrated occluded CNV vessels without damage to the neural retina (Ming et al. 2003).

2.1.9.3.4. Drusen photocoagulation

The treatment of drusen with photocoagulation in early ARM and their disappearance is not new (Gass 1972; Wetzig 1988) but conclusive data of the benefit of such treatment are still unavailable (Frennesson and Nilsson 1995; Sarks et al. 1996; Guymer et al. 1997; Little et al. 1997). It is speculated that laser treatment stimulates the macrophages which leads to the removal of cellular debris or that laser produces channels in the Bruch's membrane and thus better clearance to the choroid (Duvall and Tso 1985; Sigelman 1991). However, photocoagulation also destroys degenerated RPE cells which might lead to more debris deposition (Duvall and Tso 1985). Secondary neovascularisation and geographic atrophy could also be consequences of photocoagulation (Guymer et al. 1997; The Choroidal Neovascularisation Prevention Trial Research Group 1998; Choroidal Neovascularization Prevention Trial Research Group 2003; Owens et al. 2003; Ruiz-Moreno et al. 2003). Two large, multicentre clinical trials, the Complications of Macular Degeneration Prevention Trial (CAPT) and the Prophylactic Treatment of Macular Degeneration (PTAMD), are now underway to test whether low-intensity infrared (810-nm) diode laser treatment of drusen in patients with early ARM prevents or delays the development of late ARM (Olk et al. 1999; Figueroa et al. 2004).

2.1.9.3.5 Antiangiogenic therapy

Currently there are many ongoing studies on the use of antiangiogenic pharmaceuticals aimed to inhibit chorioretinal neovascularisation (Murata et al. 2000; Eyetech Study 2002; D'Amico et al. 2003; Eyetech Study 2003; Gillies et al. 2003; Lambert et al. 2003). An update of trials of current antiangiogenic therapies

with different drugs such as Macugen® (anti VEGF, Guyer, L), Lucentis® (anti-VEGF, Heier, J), rhuFAB® (anti-VEGF antibody fragments, Krystolik, M), (Anecortave Acetate (steroid, Slakter, JS), Oculex® (steroid, Blumenkranz, MS), Kenalog® (steroid, Aiello, LP) was given at the ARVO 2004 Minisymposium. Most of these trials are still in early phases with no results presented to date.

Angiogenesis is a complex process with a variety of components including the expression of VEGF localized in human CNVs (Kvanta et al. 1996; Lopez et al. 1996). Phase IA and II clinical studies have demonstrated that VEGF can be inhibited by anti-VEGF adaptamers (EYE001) and antibody fragments (rhuFAB® V2) intravitreally (Eyetechnology Study 2002; Krystolik et al. 2002; Eyetechnology Study 2003). Anti-VEGF adaptamers are small RNA-like molecules that bind exclusively to the 162-5-kDa isoform of human VEGF (Tucker et al. 1999). Anti-VEGF antibody fragments have a broad spectrum and high levels can be achieved intravitreally. The first published results of these clinical trials reported data on 15 subjects with subfoveal classic or occult CNV treated with anti-VEGF-adaptamer (phase 1A) showed that a single dose (up to 3mg/eye) produced improved visual acuity, decreased vascular permeability and inhibited CNV in 80% of the subjects at three months after treatment. In the second phase study, the drug was applied on three occasions at 28 day intervals with (11 subjects) or without pre-treatment with PDT (10 subjects) 5 to 10 days before. Of those subjects without PDT (n=8) and with PDT (n=10) who completed the protocol, 87.5% without and 90% with PDT had stabilized or improved vision, and 25% without and 60% with PDT had improved vision (≥ 3 lines). Similar findings have been demonstrated in the rhuFAB® V2 phase

I trial in monkeys (Krzystolik et al. 2002) with 26% showing a three line gain in vision.

The Eyetech study group reported three serious adverse events during phase II trials with myocardial infarctions in two subjects, (in one fatal) and severe depression with attempted suicide in another subject. The study group did not relate these events to the drug as the pharmacokinetic data showed plasma drug levels in these three subjects were within the mean of the values for all subjects. However, given that angiogenesis can also have an important life saving function, the systemic effect of anti-VEGF-drugs should not be underestimated. VEGF plays an important role in cardiac relaxation and blood flow and its inhibition could result in myocardial ischaemia (Harada et al. 1996). Nevertheless, to prove that the beneficial outcome after three months might not be only transient, longer follow up studies with larger sample sizes should be considered. Caution should be also applied in regards to systemic toxicity and cardiovascular adverse events (Csaky 2003).

Corticosteroids are a well known drug class with antiangiogenic effects. They reduce vascular endothelial cell extracellular matrix turnover and inhibit inflammatory cells, which might participate in a neovascular response (Folkman and Ingber 1987; Oh et al. 1999). Many studies have investigated the use of triamcinolone acetonide intravitreally (Penfold et al. 1995; Challa et al. 1998; Danis et al. 2000; Penfold 2002; Ranson et al. 2002; Gillies et al. 2003; Jonas et al. 2003; Jonas 2004). Some of these report beneficial effects over 18 months follow up while others report only short term visual improvement with progressive membrane growth. There are well known undesirable side effects of corticosteroids such as cataract and glaucoma,

which have to be considered in the use of these agents. Raised IOP occurred in up to 50% of eyes treated with higher dosages (25 mg) of triamcinolone acetonid (Jonas et al. 2003). The elevated IOP was reversible and in most cases could be controlled with topical drugs.

Recently ARM combination therapy of PDT and triamcinolone acetonide has shown promise (Spaide et al. 2003b; Rechtman et al. 2004). Although effects have been assessed in only a small number of subjects, this treatment modality could be an effective alternative. Triamcinolone is applied before or after PDT treatment and is meant to reduce the inflammatory reaction caused by PDT. Investigators have found less subretinal and subpigment epithelial oedema and better visual outcomes. Triamcinolone could also reduce retreatment frequency of PDT in future. A number of abstracts were presented at the ARVO 2004 meeting which showed similar favourable results (Rogers et al. 2004; Roth et al. 2004; Spaide 2004).

More specific drugs of the steroid family have been developed. Anecortave acetonide is a new steroid under development, its effectiveness in ARM is currently being investigated in a large multicentre trial (The Anecortave Acetate Clinical Study Group 2003). Anecortave acetonide inhibits blood vessel growth by inhibiting the enzymes urokinase plasminogen activator and matrix metalloproteinase-3 which are necessary for vascular endothelial migration (Penn et al. 2001). It has the ability to non-specifically inhibit angiogenesis independent of the initiating stimulus (Clark 1997). Inhibition of lesion growth and vision improvement (> 2 lines) with 15 mg anecortave acetate in the treated group was statistically superior to that of the placebo group ($p < 0.0017$) (The Anecortave Acetate Clinical Study Group 2003).

2.1.9.3.6 Macular surgery

Macular translocation surgery is a highly experimental procedure to stop progressive vision loss and possibly improve vision in patients with CNV. Surgery involves detaching the retina, a 360 degree retinotomy, dissection of the CNV as well as rotating (30° and 80°) and relocating the fovea away from blood vessel growth (Machemer and Steinhorst 1993). The sensory consequences of macular translocation, such as diplopia, can be compensated for by counter rotation of the globe. Stable visual acuity and function measured with the focal ERG have been reported in some subjects (Machemer and Steinhorst 1993; Eckhard et al. 1999; Abdel-Meguid et al. 2003; Terasaki et al. 2004).

Surgical excision of subfoveal CNVs has also become a possibility. However, it has been successful in ocular histoplasmosis and multifocal choroiditis but very disappointing in ARM (Lambert et al. 1992; Ormerod et al. 1994; Thomas et al. 1994). This is understandable when considering the different CNV growth pattern secondary to ARM which grows both anteriorly and posteriorly to the RPE. These membranes are difficult to excise due to interdigitation with the RPE. Further, pathomechanisms underlying ARM are thought to produce more generalized RPE dysfunction and a surgical procedure might not change this. Although subretinal removal of the CNV allowed stabilization of visual function and inhibited the development of large pseudotumor-like scars postoperatively, remaining pigment epithelial defects with choroidal atrophies have been shown to limit vision rehabilitation (Schmidt et al. 2003). Currently a large ongoing pilot trial is comparing efficacy of surgery with observation for the treatment of new or recurrent subfoveal CNVs. This study is designed to show whether submacular surgery increases the

likelihood of vision stabilization or improvement compared with observation (Submacular Surgery Trials Pilot Study Investigators 2000b; Submacular Surgery Trials Pilot Study Investigators 2000a).

Several groups have investigated the effect transplanting healthy RPE cells following CNV excision. These healthy cells might prevent, halt or slow the progress of the disease (Algvere et al. 1994; Gouras and Algvere 1996; Algvere 1997; Binder et al. 2002). Although these studies show promising results they are, however, limited to small numbers of subjects and need further investigation in large clinical trials.

As shown in this section there are a number of potential treatments for ARM in development. However, to provide the highest quality medical care for patients, treatment effects need to be assessed and documented using the most accurate and reproducible data available.

The next section of this chapter describes the multifocal electroretinogram (mfERG), its technique and applications. The mfERG is a technique for mapping localized central retinal function objectively and thus is advantageous in ARM. It is a relatively “young” method, first introduced in 1992 (Sutter and Tran 1992), and knowledge of its use and applications is still growing.

2.2 The multifocal electroretinogram (mfERG)

The mfERG is a similar response to the photopic full-field ERG, but derives responses from large numbers of small retinal areas (Fig 2.7).

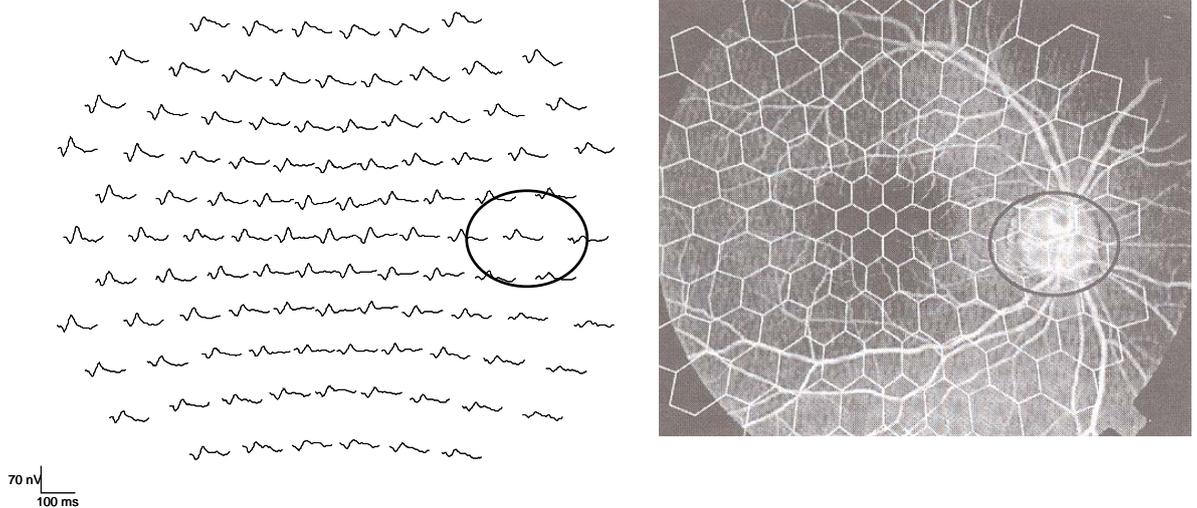


Figure 2.7. The cone-mediated mfERG waveform responses (left) derived from multiple areas of the central retina of a right eye (right). The encircled waveform indicates the blind spot area with smaller responses (left side).

By its conventional application the mfERG is thought to reflect the cone-mediated responses from the outer retinal layers. Like the full-field ERG the waveform of the mfERG consists of an initial negative (a-wave) or N1 trough, an initial positive (b-wave) or P1 peak, and an after-negative component (N2) (Fig. 2.8).

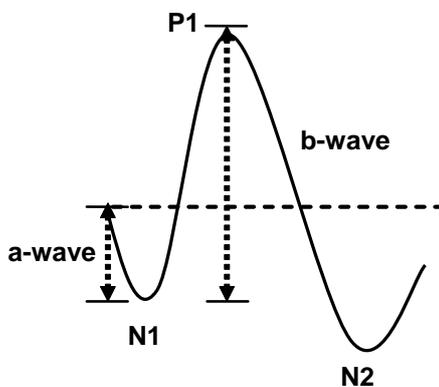


Figure 2.8. The first-order cone-mediated mfERG waveform shows a trough (N1) followed by a positive P1 and another trough labelled as N2. Similarly the full-field ERG waveform consists of a negative wave (a-wave) followed by a positive wave (b-wave).

When slowing the stimulation sequence by interleaving blank frames the mfERG can be compared with the photopic a and b-wave of the full-field ERG (Hood et al. 1997). In its original fast stimulation sequence the negative waveform of the mfERG is still thought to be shaped by the same components as the a-wave of the full-field

ERG. The positive waveform is thought to be some combination of the full-field b-wave but more likely to be modified by adaptation mechanisms (Hood et al. 1997). Two excellent reviews for basic understanding of the multifocal ERG have been published recently (Hood 2000; Seeliger et al. 2001a).

2.2.1 Techniques and recording

The cone-mediated multifocal electroretinogram (mfERG) was first introduced by Sutter and Tran (1992) and has been used in research for a number of years. It is based upon the pseudorandom m-sequence flicker stimulation technique (fast-sequence mfERG). This allows fast simultaneous recording of many local responses from the posterior pole (Sutter and Tran 1992).

2.2.1.1 Equipment and recording technique

The use of achromatic stimulation at photopic luminance allows derivation of cone-mediated retinal activity. In contrast to the full-field electroretinogram which records an overall response of the retina, the mfERG can detect localized damage of the outer retina with a high spatial resolution in a short examination time (<10 minutes). While full-field electrophysiology uses single input stimulation in combination with tools of linear system analysis, the mfERG applies spatially separated stimuli in combination with methods that permit the study of interactions between them. It uses black and white hexagons scaled with eccentricity to approximately equalize the cone-mediated responses across the stimulated fields (see also Fig 2.6), which are usually presented on a CRT display. For clinical use the most commonly used hexagonal arrays consist of 61 or 103 stimuli, covering the central 20° to 50° of the retina which allows good local resolution (central hexagon covers about 1°-2° and the most peripheral

hexagons are about 4 to 5 times larger). However, protocols with less or more hexagons showing less or more anatomical structure and physiological details have been applied (Poloschek and Sutter 2002; Scholl et al. 2002; Nagasaka et al. 2003; Shimada and Horiguchi 2003). It should be noted that higher resolution protocols also mean longer recording times which might not be suitable for clinical use (Heinemann-Vernaleken et al. 2001). Each hexagon goes through a pseudorandom m-sequence with a 0.5 probability of being either black or white, changing with each frame of the display usually every 13.3 ms (so called base interval) at a frame rate of 75 Hz. More sophisticated stimulus presentation modes have been introduced, mainly to avoid artefacts produced by CRT monitors (Zele and Vingrys 2005). These can be avoided by choosing monitor frame rates >100 Hz or using digital stimulus delivery methods (LCD) but these have not found clinical application yet (Keating et al. 2001; Zele and Vingrys 2005).

Each hexagon starts at a different point in the same m-sequence which allows analysis of the responses with a fast algorithm (Sutter and Tran 1992). While the subject views the centre of the monitor the local response is computed as the cross-correlation between the sequence and the continuously recorded ERG (Fig 2.8, left panel). It is important that the subjects are refracted to their best visual acuity at the test distance as blurring of the stimulus could reduce the contrast of local luminance modulation (Bears and Sutter 1996; Keating et al. 1996; Palmowski et al. 1999a). Similar to the full-field electroretinogram, retinal signals are recorded with corneal contact lens or thread electrodes (Fig 2.9 right panel). Skin (AgCl) cup electrodes on the forehead and earlobe serve as ground and reference electrodes, respectively.

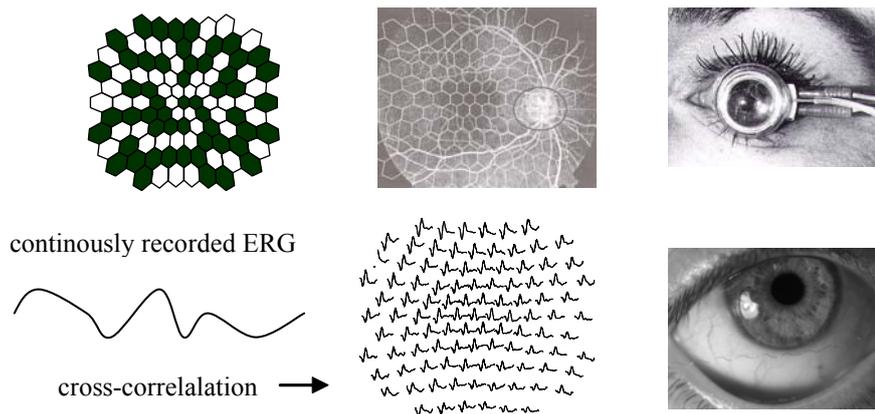


Figure 2.9. The hexagonal array of black and white hexagons which are stimulated by the same pseudorandom m-sequence (upper left field) is presented on a monitor resulting in a functional mapping of local areas of the central retina (upper middle field). A cross correlation technique produces multiple ERG records, one associated with each of the hexagons (lower middle field). The different corneal electrode types are shown on the right side (contact lens electrode, upper field; DTL thread electrode, lower field).

It should be noted that contact lens electrodes give larger amplitude responses compared to DTL electrodes (Coupland 1991; Esakowitz et al. 1993) but also less comfort to the subject.

The multi-input m-sequence stimulation technique permits the extraction of first, second and higher order response components or so-called kernels. Kernels result from cross-correlation of responses to random stimulation with auto-products of the stimulation sequence. This thesis focused on the interpretation of first-order kernels which can be obtained mathematically by adding all the records following a presentation of a white (flash) hexagon and subtracting all the records following a dark frame (black hexagon) (Fig 2.10). They are thought to represent a linear response of the retina. Given that the retina cannot be considered as a linear system, first order kernels also include nonlinearities (Sutter and Tran 1992).

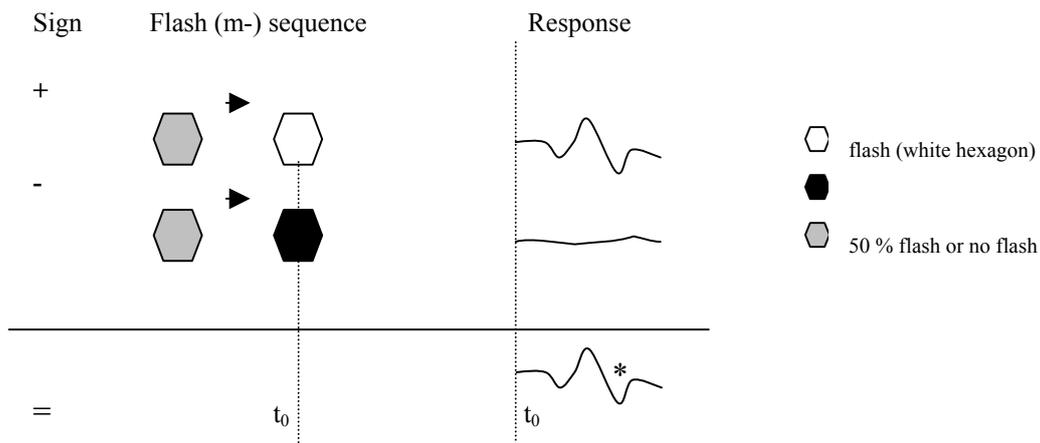


Figure 2.10. [modified from Hood et al.(2000)] The first-order kernel response (indicated with an asterisk) is derived from adding the white hexagon (or flash response) and subtracting the black hexagon (or no flash/dark frame response).

These nonlinearities are influenced by adaptation to successive flashes and reflect inner retinal cell contributions and lateral interactions (Sutter and Tran 1992). They can be better isolated by extracting higher order kernel slices from the mfERG records. For example, the first slice of the second order kernel measures the effect of an immediately preceding flash whereas the second slice of the second order-kernel measures the effect of the flash two frames away. A more extensive overview of multifocal binary kernels has been given by Sutter (2001).

Recent guidelines for basic cone-mediated mfERG recording have been published by the International Society of Clinical Electrophysiology and Vision (ISCEV) to make data comparable between different laboratories (Marmor et al. 2003). Several technical aspects with regards to stimulus delivery, electrodes, amplifiers and filter bandwidth as well as interference and noise reduction have been reviewed by Keating et al. (2000). However, research to improve the technique and the understanding of the mfERG is still ongoing.

2.2.1.2 Filter bandwidth

In practice the low signal strength of the mfERG and the high gains used also mean limiting the bandwidth to cut out baseline drift. It is common in multifocal measurements to restrict filter bandwidth in order to reduce amplifier saturation. According to the ISCEV guidelines a high-pass cutoff between 3-10 Hz and low-pass cutoff filter between 100-300 Hz can be used for the mfERG. However, it is known that restricting filter bandwidth can influence waveform shape (Keating et al. 1997). Keating et al. (1997) have strongly suggested a bandwidth as wide as possible. High-pass filter settings can have major effects on waveform shape whereas low-pass filtering presents fewer problems (Usui and Nagasaka 1994). The high-pass filter determines how fast a changing signal has to be before it is passed by the filter, thus low frequency potentials are blocked whereas high frequencies are passed. Keating et al. (1997) showed that the mfERG shape was only slightly affected in amplitude when choosing high cutoffs between 0.1 to 20 Hz in healthy subjects. However, they have also revealed that in the case of a selective reduction of the positive waveform (so-called negative ERG) which occurs typically in congenital stationary night blindness, central vein occlusion or retinoschisis, cutoffs greater than 5 Hz distorted the waveform and masked the negative ERG. Han et al. (2004a) have demonstrated higher signal-noise ratio and less noise in normal eyes when choosing a smaller bandwidth between 10-100 Hz. They have based their filter setting on the fact that the power of first-order mfERG kernel is concentrated primarily between 19-47 Hz (Bock et al. 2000). Han et al. (2004a) have detected more retinal dysfunction in diabetic eyes by choosing this filter bandwidth. They have suggested that the use of 10-100 Hz setting might also reduce inter-subject variability.

However, it might be still the choice of the laboratory which bandwidths to choose for the cone-mediated mfERG as long as it is within the recommended standard.

2.2.1.3 Fixation control

Maintaining central fixation is an important issue during mfERG recording. According to the ISCEV guidelines (Marmor et al. 2003) this should be monitored in some fashion either by direct observation of the patient or by the use of monitoring instrumentation such as an infrared eye and a fundus camera (Kondo et al. 1997; Jurklies and Sutter 2002; Moret et al. 2004). SLO techniques provide excellent fundus images and fixation observation throughout the recording session. However, the SLO and the commonly used mfERG CRT-stimulation differ in luminance levels and might cause difficulties in comparing methods (Rudolph et al. 2002; Kalpadakis and Rudolph 2003; Poloschek et al. 2003). Interestingly Rohrscheider et al. (1995) showed fixation stability even in eyes with decreased visual acuity. They observed a maximum deviation of about 2.2° for eyes with a visual acuity of 20/100 and explained fixation stability by remaining islands of foveal cone function. Moreover, it has been stated that prediction about fixation should not be based upon visual acuity as even subjects with good visual acuity can exhibit drifts by as much as 10° (Jurklies and Sutter 2002). However, it should be also noted that an array of large (fewer) hexagons causes fewer fixation problems than an array of small (more) hexagons (Keating et al. 2000).

Although eye and/or fundus monitoring would be ideal, many laboratories still work with direct observation of the subject's performance. This can be partly managed by monitoring the signal online on the computer screen during the recording. Another

method is to control fixation stability after recording which can be revealed by smaller responses around the blind spot about 15° from the central response in most subjects (see also Fig 2.7).

2.2.1.4 Pupil diameter

Pupil diameter and thus retinal illumination can have significant effects on both mfERG amplitudes and latencies. Several studies have demonstrated that pupil dilatation increases the variation in amplitude and latency (Keating et al. 1996; Keating et al. 2000; Gonzalez et al. 2004). Chan and Brown (1998) have provided values to be used for calibration of the mfERG amplitude for pupils of different sizes. They demonstrated that the first-order ERG response amplitude was directly related to the pupil size and retinal illuminance. Amplitudes increased about 100 nV between a pupil area of 8mm² to 29mm².

The influence of pupil diameter on the recording has become of increasing interest in mfERG research. Most recently the possibility to incorporate normalized data for different pupil sizes in the mfERG system was discussed between leading scientists (Sutter EE, Hood D, Frishman L and Holopigean K) at the ARVO 2004 meeting and might be available in future.

2.2.1.5 Stray light

The spatial resolution of the mfERG technique has yet to be precisely determined and is somehow limited by stray light. MfERG records together with clinical findings in retinal diseases suggest that it can detect a scotoma of less than 4° (in contrast to conventional 30° visual fields where the test locations are separated by 6°) (Yoshii et

al. 1998; Hood 2000). There are two components of stray light which need to be distinguished, a light scatter from any optics of the eyeball such as e.g. cataract (forward scatter) and a backward-scatter or a reflected light (Shimada and Horiguchi 2003). A typical example for a back-scattered stray light response is the optic disc response. Because there are no photoreceptors the responses recorded from the stimulated optic disc must be attributed to stray light (Boynton 1952). It has been explained that responses are present because rarely only one hexagon falls on the disc but parts of several hexagons, of which other parts also stimulate retinal areas (Sutter and Tran 1992; Kondo et al. 1995; Hood 2000). Usually first-order kernel (K1) optic nerve signals are smaller and delayed compared with the surrounding mfERG signals. There is evidence that lesions with high reflectance might elicit stray light-induced responses and that lowering the luminance levels reduces stray light effects (Miyake 1988; Hood 2000). In contrast it has also been reported that dim flashes can elicit large stray light responses (Hood et al. 1998b). Several studies have also suggested that background illumination suppresses the stray light effect (Hood et al. 1998b; Shimada and Horiguchi 2003). Shimada and Horiguchi (2003) investigated stray light responses from an optic disc coloboma and found that the first slice of the second order kernel (K2-1) was not affected by the stray light effect. They showed that increasing stimulus intensity affected K1 and K2-1 amplitudes equally at a luminance between 0.67 cd-sec/m^2 and 2.67 cd-sec/m^2 . The researchers concluded as the K2-1 was stray light free the increase in K1 could have not been attributed to stray light. Thus the ISCEV recommended luminances between 1.33 cd-sec/m^2 and 2.67 cd-sec/m^2 ($100\text{-}200 \text{ cd/m}^2$) have been thought to be appropriate for investigating focal lesions without excessive stray light contribution (Shimada and Horiguchi 2003).

The effect of cataract induced light scattering has been investigated in many studies (de Waard et al. 1992; Brown and Yap 1995; Arai et al. 1999; Yoshii et al. 2000; Chan et al. 2002; Tam et al. 2004). One study has suggested a central reduction by about 15-45% but increased peripheral responses by using a liquid crystal diffuser (LCD) to simulate different degrees of image degradation in healthy subjects (Chan et al. 2002). In their study a new component (P60) was detected with a latency between 55 to 65 ms which decreased with increasing eccentricity (Chan et al. 2002). This component was more apparent under minimal light scattering conditions (higher LCD electrical setting equivalent with better transparency) and was suggested to be related to retinal adaptation. In another study subjects were graded into three groups from mild to moderate cataract according to the Lens Opacities Classification System III (LOCS III) (Tam et al. 2004). Although this study has not demonstrated increased peripheral responses it has confirmed previous findings of a linear reduction of the central mfERG responses with increasing severity of cataract. Most recently the mfERG responses have been studied in subjects before and after cataract surgery (Wordehoff et al. 2004). It has been shown that the mean amplitude of the response in the central four degrees increased significantly after surgery whereas latencies remained stable.

Given the significant influence of cataract on the mfERG results it is important to perform a lens grading in an older population. A pseudophakic control group would be advantageous to differentiate between a lens and/or a retinal effect in a study with older subjects. This, on the other hand, might influence data in so far as normal conditions such as the physiological barrier between anterior and posterior segment

is disturbed. Another approach might be putting more emphasis on latency interpretation as it appears to be less effected.

2.2.1.6 Reliability and reproducibility

Given that the mfERG tests small local areas and thus is more susceptible to inter- and intra-individual variability than the full-field ERG, reliability and reproducibility have been addressed in several studies (Bears and Sutter 1996; Parks et al. 1996-1997; Kondo et al. 1998; Fortune et al. 1999; Heinemann-Vernaleken et al. 2000; Meigen and Friedrich 2002). While researchers have found an influence of the circadian rhythm on the full-field ERG with delayed latencies at night (Hankins et al. 1998) this could not be confirmed with the mfERG (Heinemann-Vernaleken et al. 2000). It has been shown that reproducibility is not significantly influenced by testing time, corneal or thread (DTL) electrodes or number of hexagons in normal eyes or eyes with macular diseases (Meigen and Friedrich 2002; Sasseville et al. 2004). However, averaging response analysis (e.g. concentric ring responses) has been demonstrated to be significantly superior to local response analysis due to better signal-noise ratio (Parks et al. 1996-1997; Meigen and Friedrich 2002). It has been shown that amplitudes of local mfERG responses are 10 times more variable than their latencies (Fortune et al. 1999). A standard deviation of about 10% of the mean amplitude for the central response has been found in three sets of three consecutive recordings in healthy subjects (Yoshii et al. 2000). Yoshii et al. (2000) showed that the mfERG was dependent on the physical condition of the subject. For example, sleep deprivation resulted in poorer mfERG reproducibility in one healthy subject.

The inter-individual variance in response density has been demonstrated to be greatest at the central fovea but is less towards more peripheral locations (Parks et al. 1996-1997; Verdon and Haegerstrom-Portnoy 1998). Less inter- and intra-individual variability has been reported for latency, with more variation reported in the vicinity of the blind spot (Seeliger et al. 1998b; Verdon and Haegerstrom-Portnoy 1998; Fortune et al. 1999; Tzekov et al. 2004). Further, the mfERG has been suggested to have a lower inter-individual response variation than the visual field (Parks et al. 1996-1997).

Recent studies with the mfERG increasingly prefer local (each hexagon) analysis methods compared to averaging methods such as averaging hexagons together in rings or hemifields (Fortune et al. 1999; Holopigian et al. 2001; Holopigian et al. 2002; Gerth et al. 2003; Bearse et al. 2004a; Bearse et al. 2004b; Han et al. 2004b). This is understandable given the technique's aim to document a disease locally. However, it should be also kept in mind that local responses can be low in signal-to-noise ratio and sometimes difficult to interpret without extensive filtering methods. Especially in untrained, inexperienced subjects in clinical routine and depending on the disease nature (more central, para or pericentral) averaging methods might be a better approach to obtain better signal-to-noise ratios.

2.2.1.7 Normal variation of the mfERG

The normal mfERG responses vary with retinal location (Miyake et al. 1989). In particular naso-temporal asymmetry for the high frequency potentials (so-called oscillatory potentials, Ops) has been reported repeatedly (Miyake 1988; Hood and Birch 1996; Rangaswamy et al. 2003). These variations can be readily seen when

slowing the mfERG sequence or decreasing the luminance and have been attributed to inner retinal cell contributions (Wu and Sutter 1995; Hood et al. 1997; Hood 2000) (see next section: 2.2.2). The standard fast sequence mfERG shows little variation but inner retinal contribution can be accentuated by lowering the mean luminance or changing the contrast (Bears et al. 1997; Hood et al. 1999c; Hood 2000). For example with mean luminances of 100cd/m^2 naso-temporal differences are also evident, although these become more apparent when the luminance is further decreased.

The entire ERG topography can be fitted with an exponential curve and shows reduced amplitudes with increasing eccentricity (Sutter and Tran 1992; Parks et al. 1996; Verdon and Haegerstrom-Portnoy 1998). The central N1P1-amplitudes are significantly larger compared to the peripheral averaged amplitudes (Sutter and Tran 1992; Nagatomo et al. 1998). Longer P1-latencies are found at the blind spot, the macula and the upper and lower borders of the stimulated field and shorter P1-latencies are evident around the macula and temporally (Seeliger et al. 1998b). The latency alterations are attributed to stray light and by-products of the stimulus. However, longer macular latencies compared to peripheral latencies and longer nasal latencies compared to temporal latencies are suggested to be due to “physiological differences” (Seeliger et al. 1998b). As with P1-latencies, the N1-latencies are longer centrally than paracentrally but longer again pericentrally (Nagatomo et al. 1998). An upper/lower retinal difference with shorter N1-latencies in the upper retina compared to the lower retina is also evident (Nagatomo et al. 1998). The P1-amplitude shows the greatest inter-subject variability. This is possibly due to inter-individual variation in cone density (Sutter and Tran 1992).

2.2.1.8 Aging effects on the mfERG

Many studies agree that there is a linear decrease in first and second order P1- and N1-amplitudes, especially centrally and an increase in the P1-latency with aging (Anzai et al. 1997; Seeliger et al. 1998b; Mohidin et al. 1999; Nabeshima 2001; Gerth et al. 2002; Jackson et al. 2002a; Nabeshima et al. 2002; Seiple et al. 2003; Tzekov et al. 2004). Seiple et al. (2003) showed that P1-amplitudes decreased by about 10.5% and N1- and P1-latencies by about 1.5% and 1.0%, respectively per decade. Most of the studies have used averaging analysis methods (central hexagons versus peripheral or concentric ring analysis) and have demonstrated a linear decrease in amplitude with age which was less with increasing eccentricity. Hemifield analysis (temporal versus nasal or upper field versus lower field) failed to show any significant aging changes (Gerth et al. 2002; Tzekov et al. 2004). Tzekov et al. (2004) have investigated the local 103 responses of seven age groups with age differences ten years apart. They found a linear decline for the amplitudes but not for latencies. Additionally it has been demonstrated that different luminance conditions (700 and 200cd/m²) as well as male and female subjects showed the same rate of age-related changes (Gerth et al. 2002).

However, there are still controversies whether aging changes in the mfERG are caused primarily by optical or neural factors (Fortune and Johnson 2002; Gerth et al. 2002; Seiple et al. 2003). Given that pupil size decreases and lens opacities increase with age, reduced retinal illuminance might impair mfERG results (see also 2.2.1.4 and 2.2.1.5). Several authors have investigated the effect of reduced luminance by choosing either neutral density filters, different pupil sizes, pseudophakes or decreased mean luminance of the screen (Brown and Yap 1995; Keating et al. 2000;

Fortune and Johnson 2002; Gerth et al. 2002; Jackson et al. 2002a). While some investigators strongly suggest that the decline with age is mainly due to preretinal optical factors (Fortune and Johnson 2002), the majority of studies suggests that there are also neural factors involved (Eisner et al. 1991; Eisner et al. 1992; Scheffrin et al. 1992; Gerth et al. 2002). The neural loss has been attributed to reduced quantal catch and altered temporal adaptation properties of the photoreceptors during aging. It is suggested that decreased luminance due to preretinal factors is possibly compensated by scattered light (large particles' scatter of the lens) and probably also by retinal adaptation (Gerth et al. 2002; Wordehoff et al. 2004). The scattered light compensation effect is thought to be highest peripherally because an increase in amplitude has been shown only centrally after cataract surgery (Wordehoff et al. 2004). While it has not been excluded that some optical factors influence age-related decrease in the mfERG amplitude, aging changes of the P1-latency in particular might be ascribed to mainly neural factors (Gerth et al. 2002).

As there is significant variation throughout aging it is necessary to compare mfERG data in diseases with data of a closely age-matched control group.

2.2.1.9 The mfERG and other vision function tests

Many studies have compared the mfERG with visual field and visual acuity measures with differing results of either good (Bears et al. 1995a; Kondo et al. 1995; Arai et al. 1998; Kretschmann et al. 1998b; Holopigian et al. 2002; Palmowski et al. 2002; Palmowski and Ruprecht 2004) or poor correlations (Yoshii et al. 1998; Odel et al. 1999; Greenstein et al. 2000b; Hood and Zhang 2000; Holopigian et al. 2001; Jurklies et al. 2002; Theodossiadis et al. 2002; R  ther et al. 2003). The visual

fields are commonly performed at lower adaptation levels, thus discrepancies between mfERG and visual fields are not surprising. There is only one study which has matched the stimulus conditions of the visual field and the mfERG to identify common mechanisms (Seiple et al. 2002). Seiple et al. (2002) have demonstrated that even under matching conditions the mfERG differed and suggested that the two tests are assessing different aspects of vision. Rohrschneider et al. (2002) found poor correlations between mfERG results and SLO fundus perimetry in subject with ARM. Chan and Brown (2000) demonstrated reduced first-and second-order kernel mfERG responses in subjects with ocular hypertension but normal visual fields. Poor correlations between the mfERG and contrast sensitivity as well as visual acuity have recently been presented in late ARM (Mackay et al. 2004).

The observations of poor correlation with other vision function tests might indicate that the mfERG assays different aspects of visual function. Different luminance and adaptation conditions have to be considered between the mfERG and psychophysical tests. This might even depend on the disease itself and how it affects the visual function. The cellular contributions to the mfERG response are diverse, mostly of postreceptoral origin and still under investigation (see next section 2.2.2). Correlating the mfERG to psychophysical function tests might help in understanding its sources and vice versa as well as the pathomechanism of a disease at cellular levels.

2.2.2 Cellular origins

2.2.2.1 Human working models based on animal studies

Recent animal studies on macaque monkeys, whose retinas are anatomically very similar to that of humans, have investigated the cellular contributions to the mfERG

waveform under photopic conditions (Horiguchi et al. 1998; Hood et al. 1999b; Hood et al. 1999c; Hare and Ton 2002; Hood et al. 2002; Rangaswamy et al. 2003). Cellular contributions (e.g. amacrine cells, ganglion cells, interplexiform cells and their connections) can be isolated by selectively blocking the activity of particular cell types pharmacologically (Hood et al. 2002). For example, the spiking activity of inner retinal cells can be blocked with intravitreal TTX (Tetrodotoxin citrate), NMDA (*N*-methyl-D-aspartic acid), GABA (γ -aminobutyric acid), glycine, PTX (picrotoxin), APB (L-2 amino-4-phosphonobutyric acid) or PDA (piperidine dicarboxylic acid). TTX prevents the generation of retinal sodium-based action potentials which are generated only in ganglion cells, some amacrine and plexiform cells. NMDA is a glutamate agonist that depolarizes cells with NMDA receptors which are also found on ganglion cells and some types of amacrine cells (Massay 1990). A combination of TTX and NMDA is thought to suppress all inner retinal contributions. However, NMDA receptors are not present on every amacrine cell and animals react differently to humans to some drugs (Hood et al. 2002). Thus additional substances such as GABA and glycine which are inhibitory neurotransmitters have been used to suppress inner retinal activity completely (Naarendorp and Sieving 1991; Vardi et al. 2000).

It has been demonstrated that intravitreal TTX and NMDA combinations produced waveforms mainly shaped by cone photoreceptors and ON and OFF bipolar cells in macaques (Hood et al. 1999c; Rangaswamy et al. 2003). By using APB, a glutamate analogue, transmission from the photoreceptors to the ON-bipolar cells can be blocked and only OFF bipolar cells and cone photoreceptors remain (Slaughter and Miller 1983). PDA which is also a glutamate analogue can be used to isolate

contributions from the photoreceptors. It blocks transmission to OFF bipolar and horizontal cells as well as to inner retinal neurons (Slaughter and Miller 1983; Naarendorp and Sieving 1991; Sieving et al. 1994).

To compare pharmacologically modified waveforms of monkeys with human waveforms, human mfERGs have been recorded under conditions as close as feasible to those for the monkeys (Hood et al. 2002). Hood et al. (2002) demonstrated that the human first-order mfERG closely resembled mfERG responses of the TTX blocked monkey. As with the monkey mfERG there was a naso-temporal difference and high frequency components (HFC), suggesting contributions generated by the inner retinal mechanisms. A third influence from the inner retina was identified which was NMDA (or GABA) sensitive and appeared as a small “shelf” in the descending part of the positive waveform (Hood et al. 2002). This shelf has been shown to be absent in glaucoma and diabetes (Hood et al. 1999c; Hasegawa et al. 2000; Hood et al. 2000).

Based on their studies in the macaque mfERG, Hood et al. (2002) developed a human working model. In this model they demonstrated that mainly depolarizing and hyperpolarizing ON and OFF bipolar cells contribute to the human first-order mfERG response. The cone photoreceptors and subtle inner retinal (spiking) activity are thought to shape the human first-order mfERG only to a small extent (Fig 2.11) (Hood et al. 2002).

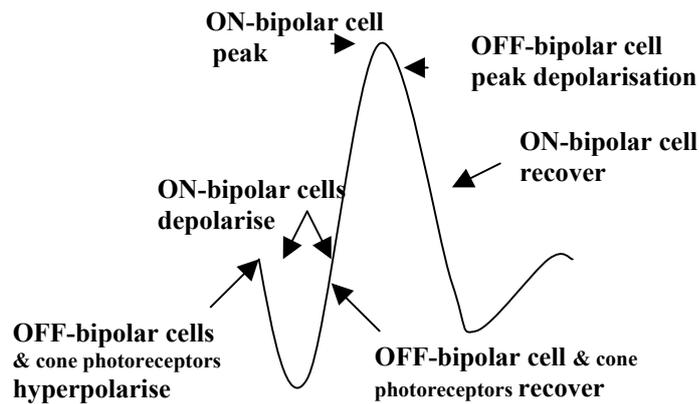


Figure 2.11. Human model of a cone-mediated first-order mfERG waveform developed by Hood et al. (2002). They found ON- and OFF-bipolars mainly contribute to the shape of the first order kernel waveform response and there is only a small contribution of the photoreceptors in the descending and ascending part of the negative waveform (inner retinal contributions not shown).

Hood et al. (1999a) demonstrated that there was a naso-temporal asymmetry due to both low frequency components (LFC) and high frequency (HFC) components with different spatial characteristics. Sutter and Bearnse (1999) have suggested that naso-temporal variations in the human mfERG might be due to an optic nerve head component. They have developed a decomposition algorithm that allowed separating the human mfERG into an optic nerve head component (ONHC) that increased in latency with increasing distance from the optic nerve head and a locally generated retinal component (RC) with latencies that were not related to the distance from the optic nerve. The local latency difference was thought to be due to propagation delays in the unmyelinated nerve fibres. This led to the hypothesis that the ONHC originates from ganglion cell fibres near the optic nerve head. It has been demonstrated that the naso-temporal asymmetry disappeared with TTX and was missing in patients with glaucoma or with optic nerve atrophy (Bearnse et al. 1995b; Bearnse et al. 1996).

The cellular source of the HFC is much more complex to understand and probably includes contributions from adaptative effects of a previous flash as well as of

following flash responses. The so-called induced components in the first order response are thought to contain information of the retinal nonlinear dynamics (Hood 2000; Sutter 2001). Hood and Birch (1996) have hypothesized that the HFC might be a compound response with various sources. Although these HFC or so-called oscillatory potentials (Ops) of the mfERG might not be necessarily from the same source as the Ops of the full-field flash ERG both show similar regional variations (Hood and Birch 1996).

Regional variations of retinal cell contributions to the mfERG have been confirmed in further animal studies by slowing (with 14 interleaved blank frames) the mfERG sequence (slow-sequence mfERG). Specifically negative and positive waveforms as well as Ops and regional variations have been better identified as the mfERG conditions became more similar to the photopic full-field ERG conditions. More oscillations have been found in the central and temporal retina than in the peripheral and nasal retina (Wu and Sutter 1995; Rangaswamy et al. 2003). Additionally Ops have been shown to be slower centrally than peripherally suggesting longer length of the cone axons in the central retina or because of a greater population of midlevel bipolar/ganglion cells with slower kinetics centrally than peripherally (Wässle et al. 1994; Rangaswamy et al. 2003). As with the fast-sequence mfERG, slow-sequence mfERG Ops have shown variation with eccentricity and naso-temporal asymmetry (Frishman et al. 2000) and recent evidence supports the hypothesis that the ONHC is involved in the naso-temporal asymmetry of Ops (Rangaswamy et al. 2003).

2.2.2.2 The retinal cell architecture and possible sources of the cone-mediated mfERG

Depending on the kernel derivation and paradigm, the mfERG has been described as reflecting various linear and nonlinear responses coming from complex circuits of different neurons throughout the retina (Sutter and Tran 1992). Thus, responses from the different photoreceptors and relations to bipolar, ganglion as well as modulatory cells such as horizontal and amacrine can contribute to greater or lesser degrees to the mfERG waveform.

In brief, there are four photoreceptor types, each of which is distinguished by a characteristic photopigment, the opsin protein that combines the photosensitive retinaldehyde (11-cis retinal) to form the visual pigments in humans. Rods contain one opsin type (rhodopsin) whereas cones contain either long- (L), medium- (M), or short- (S) wavelength specific opsins (Nathans et al. 1986; Sakmar 1998; Ebrey and Koutalos 2000) and the terminology L, M and S for cones is the more correct than red, green and blue (Pokorny and Smith 1992; Goldsmith 1994; Ebrey and Koutalos 2000).

MfERG paradigms for isolating long, middle and short wavelength sensitive cones have been introduced but have not yet found clinical application (Baseler et al. 1996; Albrecht et al. 2002). Albrecht et al. (2002) have introduced a mfERG paradigm for eliciting L- and M-cones based on the Stockman and Sharpe (1998; 2000) cone fundamentals, determined for 10° and larger viewing conditions. They investigated the normal distribution of L- and M-cones in the central 20° with this paradigm and correlated their findings with heterochromatic flicker photometry. Good correlations

were found between these two measures showing that regional differences in amplitudes and latencies were related to variations of the L- and M-cone ratios in flicker photometry. Albrecht et al. (2002) demonstrated higher P1- and N1-amplitudes centrally (5°) for the L-cones compared to M-cones. While such a difference was not found for the P1-latencies between the two cone populations, an increase with eccentricity was evident for both types. In general Hood (2000) has suggested that damage to any of the cone photoreceptor outer segments or a cone photoreceptor loss results in reduced amplitudes and a moderate delay in latencies of the conventional cone-mediated first-order kernel mfERG.

The photoreceptors synapse onto two major classes of neurons, the bipolar cells and the horizontal cells in the outer plexiform layer (OPL). In mammalian retinas several types and numbers of cone bipolar cells have been identified and only one rod specific bipolar type (Boycott and Wässle 1991; Wässle et al. 1994; Euler and Wässle 1995; Wu et al. 2000). Cone bipolar cells show physiological dichotomy; one class (ON-bipolar cells) is depolarized and the other class (OFF-bipolar cells) is hyperpolarized by a light stimulus projected onto their receptive fields (Saito 1987; Chalupa and Guenthan 2004). The axons of ON- and OFF-cone bipolar cells terminate within separate strata of the inner plexiform layer (IPL) where they make synaptic contacts with the stratified dendrites of ON and OFF ganglion cells. Hood (2000) has speculated that diseases that primarily alter synaptic transmission in the OPL causes a delay in latencies. Involvement of ON bipolar cells results in reduced mfERG first-order amplitudes with moderately delayed latencies, whereas OFF-bipolar cell loss results in larger amplitudes and possibly faster latencies.

Anatomical studies show that the IPL has highly sublaminal organisation with the axon terminals of the bipolar cells and the dendrites of the amacrine cells, horizontal and the ganglion cells. The different cell types display widely diverse morphologies, with processes stratifying at many different levels of the IPL. The portion of the IPL containing the synaptic circuitry mediating the OFF responses is called sublamina A (proximal IPL) whereas the IPL containing ON responses is called sublamina B (distal IPL) (Famiglietti and Kolb 1976; Nelson and Kolb 2003). Hood (2000) has suggested that altered synaptic transmission in the IPL might cause a small delay and possibly waveform changes in the first-order mfERG latency, but almost no effect on mfERG amplitude.

Amacrine cells are axonless interneurons (Dowling and Ehinger 1975) that synapse in the inner plexiform layer with bipolar and ganglion cells and tend to send their dendrites widely across the retina (Dowling and Ehinger 1975). More than 30 different types of amacrine cells with different neurotransmitters have been described. This variety has been thought to be necessary for adaptation to different light conditions (Wässle and Boycott 1991). Given that they synapse in the IPL, mfERG changes relating to this layer have been described to show latency delays without effects on mfERG amplitudes (Hood 2000). However, given that oscillatory potentials (OP) are thought to reflect amacrine cell function, the mfERG can measure these more distinctly by using a slow-flash paradigm (Wu and Sutter 1995; Hood et al. 1997; Bearse et al. 2000; Rangaswamy et al. 2003). Several studies have shown delayed OP latencies or reduced OP amplitudes in diabetic retinopathy (Kurtenbach et al. 2000; Onozu and Yamamoto 2003; Bearse et al. 2004a). In particular, Bearse et

al. (2004a) demonstrated that local first- and second-order kernel mfERG OPs were delayed in subjects with early, non-proliferative diabetic retinopathy.

Research on horizontal cells is still ongoing and shows some controversies (Bears et al. 2004a). There are different types of horizontal cells with specific dendrites contacting only cones or cones and rods (Kaiser and Boynton 1996) and there is controversy about selective chromaticity (Wässle and Boycott 1991; Ahnelt and Kolb 1994; Twig et al. 2003). However, chromaticity (C-type) horizontal cells have been described in several species but their major functional role is still not clear (Twig et al. 2003).

The largely horizontal organisation permits horizontal cells to mediate connections between the receptors, bipolar and ganglion cells. While their cell bodies are found in the inner nuclear layer, the ramifications of their dendrites and axons contribute to the IPL and OPL. Damage to horizontal cells is mainly thought to cause a delay of latencies in the mfERG due to transmission rather than an amplitude alteration (Hood 2000).

The functional segregation of ON- and OFF- bipolar cell inputs to the IPL has been established across many species. This linkage of morphology with physiology is continued in all classes of ganglion cells (Nelson and Kolb 2003) and the difference between ON and OFF ganglion cells is the type of cone bipolar input they receive. ON- and OFF bipolar cells innervate ON and OFF center ganglion cells respectively, by expressing different glutamate receptors (Fain et al. 1983; DeVries 2000; Chalupa and Guenthan 2004). Several neurotransmitter systems, including glutamatergic,

acetylcholinergic, GABAergic, and glycinergic systems, might act together to modulate the ganglion cells dendritic refinement.

Colour-coding ganglion cells fall into two major physiological classes: the red-green opponent cells, which receive antagonistic input from M- and L-sensitive cones, and the blue-yellow opponent cells, which receive input from S-sensitive cones, opposed by combined M- and L-cone input (Dacey and Lee 1994). In the primate retina, colour specific ganglion cells with small receptive fields project to the parvocellular layers of the lateral geniculate nucleus (LGN) whereas non-colour specific large concentric receptive field ganglion cells project to the magnocellular LGN layers (De Monasterio and Gouras 1975; Kaplan and Shapley 1986).

Several neurochemically distinct retinal circuits and several retinogeniculate classes of ganglion cells indicate that there might be more than two pathways (Rodieck and Watanabe 1993; Dacey and Lee 1994; Hendry and Calkins 1998). For example, a separate type of ganglion cell, displaying a center only ON-response to S-cone stimulation has been identified (Dacey and Lee 1994). These ganglion cells are thought to have separate dendritic branches in the ON and OFF sublamina of the IPL and show exclusively S-cone ON responses. Additionally a koniocellular pathway occupying regions ventral to each of the magnocellular and parvocellular layers has been identified in some species including macaques (Hendry and Yoshioka 1994). This pathway is thought to carry mixed-cone responses but also S-cone ON cells in primates (Hendry and Yoshioka 1994; Martin et al. 1997).

Damage to ganglion cells is thought to be hardly reflected in the conventional (high luminance, first-order kernel) mfERG (Hood 2000) although damage due to glaucoma might effect amplitudes, latencies or waveforms (Chan and Brown 1999; Hasegawa et al. 2000; Fortune et al. 2001; Palmowski and Ruprecht 2004). In particular, Sutter and Bearnse (1995; 1999) have introduced the optic nerve head component which presents evidence for a response of the optic nerve which is missing in glaucomatous subjects. Several studies using different mfERG paradigms such as lower contrast and luminance mfERG displays (Bearnse and Sutter 1998; Hood et al. 1999c; Sutter and Bearnse 1999; Hood et al. 2000; Palmowski et al. 2000; Raz et al. 2002) or global flash paradigms (Sutter and Bearnse 1998; Sutter et al. 1999) suggest that the use of such different ERG protocols better detects ganglion cell damage in glaucoma.

Different ON and OFF pathways for cones and rods have been described. In the light adapted state cone photoreceptors release glutamate and activate specific glutamate receptors of ON-cone bipolar cells which hyperpolarize. Light activation stops the release of glutamate by the photoreceptors and increases conductance of ON-bipolars which release glutamate themselves and cause depolarisation of the ON retinal ganglion cells. During this period OFF-bipolar cells have reduced conductance by light onset and are maintained in a depolarised state by the cone photoreceptors' continuous glutamate release (Chalupa and Guenthan 2004). In contrast different ON/OFF pathways are functional in the dark adapted state and are thought to be multiple (Sharpe and Stockman 1999). Unlike cold-blooded vertebrate rods, mammalian rods are thought to synapse with a single type of bipolar cell which depolarizes following light stimulation (the so-called ON type) (Wässle and Boycott

1991). The first pathway which has been identified is transmission of rod photoreceptor signals to rod (ON) bipolar cells. Rod bipolar cells terminate close to ganglion cells bodies but make no direct output synapses (Kolb and Framiglietti 1974) and contact only amacrine II cell processes (ON rod bipolar and AII amacrine pathway) (Wässle and Boycott 1991; Sharpe and Stockman 1999; Chalupa and Guenthan 2004). Amacrine II cells form gap junctions with ON-cone bipolar cells as well as inhibitory synapses with OFF-cone bipolar cells and OFF ganglion cells (Sharpe and Stockman 1999). Thus rod bipolar cells can simultaneously excite and inhibit ON and OFF ganglion cells (Sharpe and Stockman 1999). Rod-cone gap junctions have been described to form a second pathway so that rod signals have access to ON and OFF cone bipolars and thence to ON and OFF ganglion cells (Kolb and Nelson 1983). Whether a third or direct rod to OFF bipolar pathway exists or not still needs to be investigated but there is strong evidence that it only exists in rodents, but is absent in humans (Sharpe and Stockman 1999).

Given the large number of functions of different neurons and the variety of possible connections and circuits, it is understandable that the cone-mediated mfERG can provide a large pool of summed and complex information which is still not fully investigated. An attempt to identify different cellular contributions to the human cone-mediated mfERG has been made by blocking various neurons pharmacologically in animals.

While these studies all have investigated the cone-mediated contribution to the mfERG waveform, no studies have used pharmaceuticals to better understand the cellular contributions to the rod-mediated mfERG. However, the mfERG paradigm

can be manipulated to favour rod over cone responses by dark adaptation, slowing the stimulation rate and using low luminance and short wavelength stimulation.

2.2.3 Rod-mediated mfERG

The rod-mediated mfERG has proven to be difficult to obtain (Wu and Sutter 1995; Baseler et al. 1996; Hood et al. 1998b; Nusinowitz et al. 1999). The rods lack directional sensitivity and thus are especially sensitive to stray light (Crawford 1972). It is not surprising that with increasing lens opacification an older population might produce more stray light (Hood et al. 1998b). Several studies which isolated focal rod-mediated responses with brief, weak focal flashes (from 5° to 40° in diameter) have tried to overcome stray light using different methods (Horiguchi et al. 1991; Nusinowitz et al. 1994; Sandberg et al. 1996). For example Sandberg et al. (1996) have isolated the stray light component to a focal flash by presuming that it was equivalent to the full-field response to a dimmer flash. They subtracted the matching full-field response from the mixed response to the focal flash to elicit a focal flash response. Different approaches have been used to isolate rod-mediated responses with the mfERG. Either fast sequence mfERG and isoluminant stimulation (under same photopic but different scotopic contrasts) (Baseler et al. 1996) or slow-sequence mfERG under mesopic conditions (Wu and Sutter 1995) have been applied. Hood et al. (1998b) were the first to compare the mfERG rod-mediated responses to the full-field rod-mediated ERGs. They slowed the stimulation sequence by inserting 14 blank frames between stimulus frames. For the full-field ERG the 14-frame mfERG condition was simulated with multiple full-field flashes. After 40 minutes of dark adaptation they obtained records from a stimulus array of 61 equally sized hexagons (Figure 2.12).

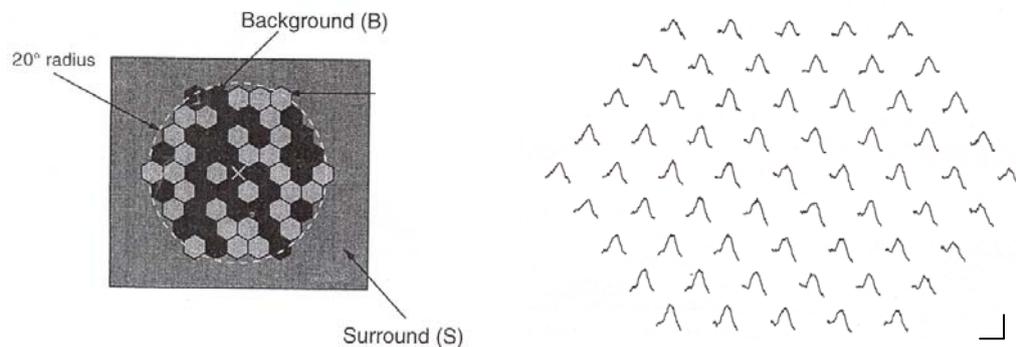


Figure 2.12. [modified from Hood et al. (1998b)] The 61 hexagonal stimulus array for the rod-mediated mfERG with reduced luminance conditions for background and surround (left). The waveform responses for 14 frame stimulus conditions of a young experienced subject are shown on the right side (14 minutes recording and contact lens electrode). The horizontal and vertical bars indicate approximately 200 ms and 0.2 μV , respectively.

The larger sized hexagons were chosen for a better signal-to-noise ratio, and were not scaled for eccentricity because of the relatively uniform distribution of the rods in the central retina. By using these recording conditions, mfERG rod-mediated responses were about 25% smaller than the full-field responses. This was mainly attributed to the smaller number of stimulated rods in the centre of the retina and to adaptation effects caused by successive flashes.

A range of different luminance conditions (-1.0 to 1.7 log scotopic trolands) for flashes, background and a surround of -0.8 log scotopic trolands were chosen to lower stray light influence. Hood et al. (1998b) found a difference between the full-field and mfERG waveforms with an early focal rod-mediated response and a later stray light response in the mfERG. The late response was attributed to the light falling outside the retinal area covered by the array of hexagons and was typically larger and slower than the local rod-mediated response. The late response decreased in amplitude with increased surround intensity (from no surround to 2.0 log scotopic trolands) and dimmer flashes (between -0.1 log scotopic trolands and 1.2 log

scotopic trolands). While an increased flash intensity had relatively little effect on the amplitude of the early (or focal rod-mediated) response, lower flash intensities eliminated the stray light. Thus Hood et al. (1998b) recommended that the light conditions (surround, background and flash) to be set as low as possible to obtain reasonable rod-mediated responses.

Based upon comparison with the 14-frame full-field ERG waveforms Hood et al. (1998b) suggested that the rod-mediated mfERG was mainly generated by the rod bipolar cells. Both protocols exhibited mainly a positive waveform with a very small negative component (a-wave) which is a photoreceptor contribution. In previous work it had been shown that negative postreceptoral potentials contributing to the a-wave were more affected by adaptation (e.g. due to successive flashes) than were the positive responses (Hood and Birch 1996).

Nevertheless, despite attempts to minimize stray light, the local resolution of the rod-mediated mfERG is still questioned (Hood et al. 1998b; Jackson et al. 2004). The responses are possibly coming from a retinal area that is larger than the region covered by a single hexagon because of stray light (Hood et al. 1998b). Hood et al. (1998b) chose relatively large hexagons (5°) which might have contributed to a limited local resolution (Hood et al. 1998b). However, smaller hexagons would also require extended recording periods to obtain a reasonable signal-to-noise ratio (Hood et al. 1998b).

Hood et al. (1998b) recommended a shorter protocol for clinic with fewer (3) interleaved blank frames (3F) (Figure 2.13). Signals were amplified with cut offs at 1 and 300 Hz because of the slower rod signal characteristics.

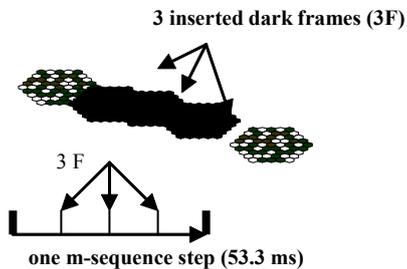


Figure 2.13. Schematic representation of the three inserted blank frame (3F) condition suggested by Hood et al. (1998b) for clinical use. Each step in the stimulus m-sequence was four frames long (53.3 ms, frame transition every 13.3 ms). In the first m-frame each location had an equal probability of flashing between black and white. In the next three frames the entire stimulus area was dark.

Nonetheless they stated that optimal recording conditions still needed to be investigated and might depend on the particular experimental setup and on the patient population. So control experiments to determine optimal stimulus conditions were recommended (Hood et al. 1998b). Moreover, the rod-mediated mfERG stimulus recommendations presented by Hood et al. (1998b) were based upon a small number of young and experienced subjects ($n=2$). Only two other studies using the recommended (3F) clinical paradigms have been performed in young subjects ($n=7$ and 8) with hereditary diseases (retinitis pigmentosa and progressive cone dystrophy) (Holopigian et al. 2001; Holopigian et al. 2002) (Fig 2.14).

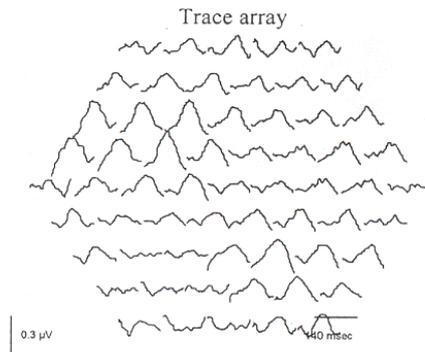


Fig 2.14. [adapted from Holopigean et al. (2002)]. The trace array of the rod-mediated mfERG from a subject aged 29 years with progressive cone dystrophy using Hood et al.'s (1998b) clinical 3F protocol.

Given the challenging procedure (Hood et al. 1998b; Jackson et al. 2004) no study has previously measured the rod-mediated mfERG in older, inexperienced subjects or in early or late ARM.

2.2.4 Analysis methods

Currently three analysis methods are available: conventional amplitude and latency (Sutter and Tran 1992), the scalar product (Sutter and Tran 1992) and the computer fitting (Hood and Li 1997) analysis. The two latter methods are calculated from the entire waveform response and are thought to be less susceptible to noise (Sutter and Tran 1992; Hood et al. 1998b). The conventional method is ideal for large responses such as those displayed by the full-field ERG (b-wave amplitude about 150 μV). Nevertheless, for the smaller responses averaged normal templates that are specific to the local abnormality are advantageous (Hood and Li 1997; Fortune et al. 1999; Hood 2000). Templates are crucial for analysis of the rod-mediated mfERG responses which are smaller in amplitude (Hood et al. 1998b).

MfERG responses can be analysed locally for each hexagon or averaged in rings, groups, quadrants or hemifields. However, the method which might be chosen depends on the disease and its underlying pathogenesis. Concentric or annular diseases such as maculopathies might be best detected with concentric ring methods or averaging central hexagons together (Seeliger et al. 2001a). The local analysis can be considered for detecting small deficits although there is a limitation to the spatial resolution due to stray light (see also 2.2.1.5). However, the averaging analysis has the disadvantage of losing local information whereas local analysis shows greater variability and poorer signal-to-noise ratio (Meigen and Friedrich 2002; Seiple et al. 2003).

2.2.4.1 Conventional analysis

The P1-amplitude (N1P1) is measured from the most negative trough (N1) to the most positive peak (P1) whereas N1-, P1- and N2-latencies are the time measurements taken from the onset of the stimulus to N1, P1 or N2 (Figure 2.15).

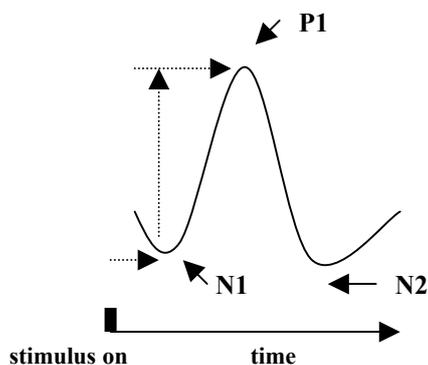


Figure 2.15. The P1-amplitude is measured from the most negative trough (N1) to the most positive peak (P1). The N1-, N2- and P1-latencies are taken from the onset of the stimulus to the first and second troughs (N1, N2) and the first peak (P1).

2.2.4.2 Scalar product

The scalar product method relies on comparison with a normal or standard waveform template. It gives a measure of deviation from an ideal response and is an effective

measure of waveform shape (Sutter and Tran 1992). It is formed by multiplying corresponding points between a template waveform and the recorded waveform. Each multiplication is then added to give a single number. This value is divided by the area of each of the particular hexagonal elements and gives a response density in nanovolts per square degree (nV/deg^2) (Sutter and Tran 1992). Small scalar products are generated when subjects and standard waveforms differ either in amplitude or timing. The advantage of this method is that it provides an amplitude with good noise immunity (Sutter and Tran 1992). However, care must be taken when more subtle changes with latencies are expected as the scalar product method has been shown to be less sensitive (Keating et al. 2000).

2.2.4.3 Computer fitting method

Hood et al. (1997) introduced a method which allows detection of small signals and precise measures of both amplitude and latency. This method also uses a global template or multiple templates derived from the waveform responses of a representative control group. The template is fitted against the subjects' records by varying three parameters. The a-parameter (a-scale) scales the amplitude at each point and the t-parameter (t-scale) scales the time vector by a single value (Fig 2.16). The third parameter shifts the template vertically to account for small changes in baseline (so called "offset") and is of less importance in interpreting the data.

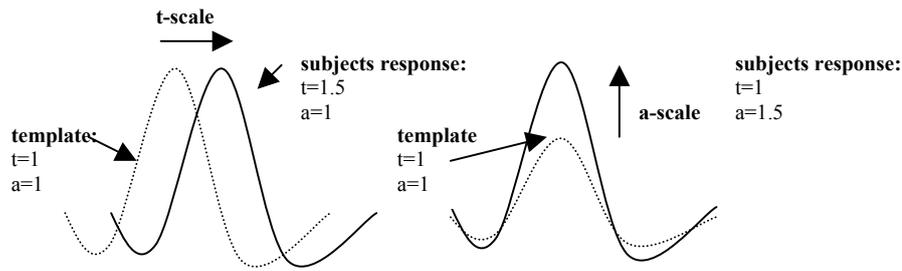


Figure 2.16. The computer fitting method uses a template based upon a representative group or subject ($a=1$, $t=1$) which is scaled (stretched) in a time (t -scale, left side) and amplitude (a -scale, right side) dimension to find the best fit to a subject's response.

For each local hexagonal response the template is scaled (or stretched) along both amplitude and time dimensions in a least-squares fitting procedure to find the best fit. A statfit value, which is the least squares statistic minimized during the fitting routine, provides a measure of the quality of a fit. A perfect fit to the template produces a value of 0.0 whereas a value of 1.0 indicates that the template fit is no better than the mean of the data. The statfit value can also be related to a measure of a signal-to-noise ratio, so a goodness of fit (criterion) value should be established for deciding which signals should be considered as responses or as noise (Hood and Li 1997). Several studies have set the goodness of fit value to less than 0.75 based on examining the waveform responses (Hood and Li 1997; Holopigian et al. 2001; Holopigian et al. 2002). Currently two other studies have successfully applied this method for analysing the small and low signal-to noise-ratio rod-mediated mfERG responses in hereditary diseases (Holopigian et al. 2001; Holopigian et al. 2002).

2.2.5 The multifocal ERG and diseases of the retina

Conventional mfERG tests (cone-mediated, first-order kernel analysis) have been used to document a number of hereditary diseases, e.g retinitis pigmentosa (Chan and Brown 1998; Hood et al. 1998a; Seeliger et al. 1998a; Holopigian et al. 2001;

Seeliger et al. 2001b), Stargardt disease (Kretschmann et al. 1998c), Best's disease (Scholl et al. 2002; Palmowski et al. 2003), central areolar choroidal dystrophy (Feigl and Haas 2001; Nagasaka et al. 2003), occult macular dystrophy (Piao et al. 2000), congenital stationary night blindness (Kondo et al. 2001) and X-linked retinoschisis (Piao et al. 2003). It has also been successfully applied to the documentation of a wide range of acquired diseases, involving the central retina, for example diabetes (Palmowski et al. 1997; Greenstein et al. 2000b), glaucoma (Chan and Brown 2000), central or branch retinal vein occlusion (Dolan et al. 2003; Hvarfner et al. 2003), central serous chorioretinopathy (Marmor and Tan 1999), toxic effects of hydroxychloroquine and vigabatrin (Kellner et al. 2000; Besch et al. 2002; Penrose et al. 2002; Maturi et al. 2004) and in inflammatory diseases (Arai et al. 1998; Kretschmann et al. 1998a; Feigl et al. 2002). Reviews of the diseases where the first-order mfERG has been clinically applied have been given by Kretschmann et al. (2000) and Seeliger et al. (2001a) and a list of publications in various fields with the mfERG can be found on the website <http://www.electro-diagnostic.com/EDIPub.html>.

The predictive capability of the cone-mediated mfERGs has just started to raise more interest. In particular, the first-order cone-mediated mfERG latencies have been demonstrated to be sensitive values of early impairment (Hasegawa et al. 2000; Gerth et al. 2003; Han et al. 2004b). In a most recent study, Han et al. (2004b) showed delayed latencies a year before diabetic retinopathy was manifest funduscopically. Impairment of mfERG measures have been found in subjects with ocular hypertension and normal visual fields (Chan and Brown 2000). However, the predictive ability of the fast-sequence mfERG first-order kernel responses in

glaucoma still needs to be investigated (Palmowski et al. 2000; Palmowski and Ruprecht 2004).

The conventional mfERG has been widely used for documenting treatment effects, such as of intraocular surgery or laser therapy. Several studies performing epimacular membrane or macular hole surgery as well as retinal transplantation have been documented well with the conventional mfERG (Radtke et al. 1999; Moschos et al. 2001; Apostolopoulos et al. 2002; Horio and Horiguchi 2004). Laser treatment effects in diabetes have revealed impaired retinal function in areas greater than the treated areas and latencies rather than amplitudes were preferentially affected (Greenstein et al. 2000a). The effects of intravitreal triamcinolone acetonide injection on outer retinal function in macular oedema due to central vein occlusion and diabetes have been recently documented with the mfERG (Park et al. 2004). As new surgery techniques and treatment modalities appear, objective documentation of function has become a necessary part of treatment studies.

2.3 Age-related macular degeneration and the multifocal ERG

Given that ARM is a central retinal disease, the mfERG is valuable for objectively assessing central retinal function. There are several studies which have shown an impairment of the conventional cone-mediated mfERG in early and late ARM (Jurklies et al. 1999; Martinson et al. 1999; Palmowski et al. 1999b; Huang et al. 2000; Jurklies et al. 2000; Martinson 2000; Martinson et al. 2000; Heinemann-Vernaleken et al. 2001; Palmowski et al. 2001; Gerth et al. 2003). A recent abstract report presented at ARVO 2004 demonstrated that delayed latencies predicted

subsequent vision loss due to progression from early ARM to late ARM (Watson and Haegerstrom-Portnoy 2004). In this study (n=13) cone-mediated mfERGs at baseline were compared after 3.5 years. The seven subjects with delayed latencies at baseline developed progressive visual acuity loss (greater than 2 lines) and either wet (4) or dry (3) AMD after 3.5 years. Of the six other subjects who retained stable visual acuity, only one showed delayed mfERG latency at baseline. Watson and Haegerstrom-Portnoy (2004) concluded that the P1-latency of the mfERG predicted visual acuity loss due to late ARM but a larger population size was needed to give their results more statistical power.

In early ARM inconsistent results have been found regarding latency and amplitude and which measure is first affected. This might be partly due to differing study designs and analysis methods (Li et al. 2001; Gerth et al. 2003). For example some studies have limited numbers of subjects or include both eyes of one subject (Palmowski et al. 1999b; Huang et al. 2000; Heinemann-Vernaleken et al. 2001; Gerth et al. 2003), others have control groups of younger age than the ARM group (Huang et al. 2000; Palmowski et al. 2001), some used different analysis methods (Li et al. 2001; Gerth et al. 2003) or did not grade funduscopy changes according to international grading systems (Huang et al. 2000; Li et al. 2001; Palmowski et al. 2001).

Currently four studies (Palmowski et al. 2002; Jiang et al. 2003; R  ther et al. 2003; Lai et al. 2004) and several reports in abstract form (Bunse et al. 2001; Prunte et al. 2001; Fredette et al. 2003; Fredette et al. 2004; Tufail et al. 2004) have measured the cone-mediated mfERG after one PDT. Most of these studies show stabilisation of

objective function. While Palmowski et al. (2002) and Ruether et al. (2003) did not test their subjects at consistent time intervals after treatment (mean seven and six weeks after PDT, respectively), the other two studies have divided treatment effects into short- and long-term effects.

It is important to differentiate between reversible short-term effects (probably inflammatory) and longer-term “real” treatment effects on outer retinal function (Rogers et al. 2002; Schmidt-Erfurth et al. 2002b; Michels and Schmidt-Erfurth 2003). Jiang et al. (2003) found no significant short term impairment three days and one week after treatment. In contrast Lai et al. (2004) detected a transient reduction in amplitude and delay in latency after four days which recovered successively to pre-treatment values after two and four weeks.

Two of the PDT studies with the mfERG have also included CNVs secondary to other diseases such as myopia (Rüther et al. 2003; Lai et al. 2004) and idiopathic CNVs as well as central serous chorioretinopathy and polypoidal choroidal vasculopathy (Lai et al. 2004). These results should be considered cautiously as CNVs found in young subjects, possibly underlie a different pathomechanism and also may have a different reaction to the treatment. Usually a number of PDT treatments (on average 6 within the first two years) are necessary in AMD (see also 2.1.3.2) but no study has investigated the treatment effects on outer retinal function with the mfERG in AMD after several PDTs.

2.4 Conclusion

In conclusion the pathogenesis of ARM is still unclear and is thought to be based on several factors (e.g genetic predisposition together with environmental factors) finally resulting in functional impairment and vision loss due to photoreceptor death. It has become a major public health goal to develop preventative measures, detect ARM early and better monitor treatment outcomes in late ARM. This thesis focuses on understanding the retinal dysfunction which occurs in ARM with the aim to provide insight into the pathways and mechanisms affected in ARM. The mfERG is an objective tool and measures local responses originating from various retinal cells. An improved understanding of the sites involved in ARM by correlating the mfERG results with well known functional tests might be one approach to a better understanding of the pathomechanisms in ARM. Investigating the earliest involved cell population, thought to be the rods, by monitoring rod-mediated function objectively over a period of time might be another approach. These topics form the main goals of the studies reported in the following three chapters 3, 4 and 5. Chapter 6 finally investigates the usefulness of the mfERG for treatment strategies in photodynamic therapy (PDT) in a pilot study. PDT is the only treatment in wet AMD which is thought to target the CNV selectively. However, little is known of how it affects the function of the overlying neurosensory retina and research on this therapy is ongoing (Schmidt-Erfurth et al. 2004).

CHAPTER 3

Cone-mediated multifocal electroretinogram in early age-related maculopathy and its relationships with subjective macular function tests

3.1 ABSTRACT

3.1.1 Purpose. To investigate the cone-mediated multifocal electroretinogram (mfERG) and subjective function in early age-related maculopathy (ARM).

3.1.2 Methods. Seventeen subjects with early ARM with visual acuity (VA) of 6/12 or better and 20 age-matched control subjects were examined. We assessed cone-mediated mfERGs, high and low contrast distance VA, near VA, low luminance VA, contrast sensitivity, saturated and desaturated Panel D-15 and visual fields (mean sensitivity). The mfERG responses were analysed by comparing central-overall (method 1) and superior-inferior (method 2) ratios.

3.1.3 Results. The cone-mediated mfERG did not discriminate between the groups while colour vision (tritan deficiency), contrast sensitivity and HC- and LC-VA showed significantly reduced responses for the early ARM group compared with the control group ($p \leq 0.01$). The mfERG first-order kernel responses correlated significantly with the desaturated D-15 in both methods ($r = -0.5$, $p \leq 0.05$). Fundus grading was not correlated with the mfERG measures.

3.1.4 Conclusion. Although the mfERG correlated significantly with the desaturated D-15 in early ARM suggesting it operates at a sensitive level, it failed to discriminate between the control and ARM groups. In our sample the subjective function measures were more sensitive than the mfERG measures.

3.2 KEYWORDS

Age-related maculopathy, early ARM, mfERG, multifocal electroretinogram.

3.3 INTRODUCTION

Early age-related maculopathy (ARM) influences the quality of vision as assessed by many psychophysical functions. Many studies have shown that psychophysical tests of contrast sensitivity, colour vision, dark adaptation and glare recovery, which assess both cone and rod-mediated function, can be impaired before high-contrast visual acuity deteriorates (Collins 1986; Brown and Lovie-Kitchin 1987a; Eisner et al. 1987a; Sunness et al. 1988; Collins and Brown 1989a; Haegerstrom-Portnoy and Brown 1989; Sunness et al. 1997; Owsley et al. 2001; Phipps et al. 2003). Given the subjective nature of these tests an objective follow up of visual function might be more reliable (Parks et al. 1996-1997; Heinemann-Vernaleken et al. 2000; Meigen and Friedrich 2002). Further, in routine clinical examination distance visual acuity is often the only clinical parameter used to quantify vision which means that discrete functional deficits might not be detected.

The conventional cone-mediated multifocal electroretinogram (mfERG) developed by Sutter and Tran (1992) can give valuable objective information about the local functional condition of the central and paracentral retina (Bears et al. 1994; Kondo

et al. 1995; Bearse and Sutter 1996; Palmowski et al. 1997; Kretschmann et al. 1998c; Seeliger et al. 1998a). It is a powerful tool especially in photoreceptor diseases with slight or no visible fundus changes such as central serous chorioretinopathy (Odel et al. 1999; Chappelow and Marmor 2000; Greenstein et al. 2000b). Several studies of hereditary diseases of the retina and ARM indicate a greater involvement of the retina beyond the ophthalmoscopically visible, affected areas (Kretschmann et al. 1998c; Martinson et al. 1999; Palmowski et al. 1999b; Jurklies et al. 2000; Piao et al. 2000; Li et al. 2001; Gerth et al. 2003). Marmor (2002) strongly suggested that the mfERG should be used for objective follow up in maculopathy, rather than being used when damage is already seen ophthalmoscopically. Especially in the early course of ARM when pathology is not clearly evident, the mfERG may be helpful in detecting changes.

Impaired responses in the mfERG affecting either amplitude, latency or both have been shown in different stages of ARM (Palmowski et al. 1999b; Huang et al. 2000; Jurklies et al. 2000; Martinson et al. 2000; Li et al. 2001; Palmowski et al. 2001; Gerth et al. 2003). Li et al. (2001) and Gerth et al. (2003) suggested that the first order kernel implicit time is a sensitive indicator of functional changes in early ARM. Palmowski et al. (2001) found the first and second-order kernel P1-amplitudes to be affected first in early ARM. Different studies have found variable degrees of agreement in correlations between the mfERG, visual acuities and visual fields depending on the disease studied. Palmowski et al. (2002) showed good local correspondence between the mfERG and the visual field after photodynamic laser therapy in late ARM. Holopigian and colleagues (2001; 2002) also demonstrated good correlations between visual field sensitivities and mfERG latencies in subjects

with retinitis pigmentosa. Odel et al. (1999) showed the mfERG to be more sensitive than the central sensitivity of threshold perimetry in occult macular dystrophy with delayed peak latencies across a 50 degree field but normal peripheral thresholds on perimetry. Jurklies et al. (2002) found correlations between the response densities of the mfERG and visual acuities only in some subjects with subfoveal choroidal neovascularisation due to ARM and secondary to myopia. There have been no studies considering correlations between mfERG and a range of psychophysical tests in early ARM.

Our main goal was to correlate the mfERG and a battery of psychophysical vision function tests in early ARM to find a possible relationship between subjective and objective function tests. Further we investigated if the mfERG correlates with morphological fundus changes.

3.4 MATERIALS AND METHODS

3.4.1 Subjects

Thirty-seven subjects were selected from the Optometry Clinic at the Queensland University of Technology, Brisbane and from the practices of local Ophthalmologists. Inclusion criteria were: visual acuity of 6/12 or better, no history of ocular diseases (glaucoma, diabetes, and uveitis) or ocular surgery, no spherical refractive error greater than 5 dioptres, no congenital colour vision deficiency and no intake of digitalis or chloroquine derivatives. Seventeen subjects (9 females and 8 males, age range 63-75 years, mean 70 years) formed the early ARM group (17 eyes). Twenty age-matched subjects (10 females and 10 males, age range 58-72 years, mean 68 years) with normal fundi and visual acuity of 6/6 or better formed the

control group (20 eyes). Subjects underwent clinical examination, slit-lamp and fundus photography and if necessary fluorescein angiography to exclude choroidal neovascularization (CNV) in both eyes. All subjects who enrolled in the study gave written informed consent and the tenets of the Declaration of Helsinki and the requirements of the University Human Research Ethics Committee of the Queensland University of Technology were followed.

3.4.2 Slit-lamp and fundus photography

Slit-lamp photographs for grading any crystalline lens changes were taken using a Nikon photo slitlamp. The clarity of the lens was graded by using the retro-illuminated templates of the Age-Related Eye Disease Study (AREDS) clinical lens standards (Age-Related Eye Disease Study Research Group 2001b). Subjects with posterior subcapsular cataract, cortical and nuclear opacities graded higher than one were excluded. Colour fundus photographs (Zeiss Jena Mydriatic Fundus camera) of the central 30 degrees of the posterior pole (centred on the fovea) were taken and retinal changes were graded independently by two experienced observers (BF, PS) using a set of the Wisconsin age-related maculopathy standards with example photographs and a grid (AREDS Reading Center, University of Wisconsin, Madison) (Klein et al. 1991; Age-Related Eye Disease Study Research Group 2001a). The drusen extent as well as RPE abnormalities were graded based upon the fundus photography. One observer (PS) was masked to the subjects' functional results and there was agreement in all subjects with judgments of the other investigator (BF, not masked).

Table 3.1. Fundus appearance and grading levels for our subject's selection adapted from the AREDS system

<u>Drusen size μm (LI)</u>	<u>groups</u>
<63 μm and total area <125 μm	1
<u>presence of one or more (LII)</u>	
a. Drusen max size > 63 μm but < 125 μm	1
b. Drusen total area > 125 μm	1
c. Retinal pigment epithelial abnormalities consistent with AMD, defined as one or more of the following in the central or inner subfield	2
1. Depigmentation present	2
2. Increased pigment > 125 μm	2
3. Increased pigment present and depigmentation at least questionable	2
<u>presence of one of the following(LIII)</u>	
a. Drusen max size > 125 μm	1
b. Drusen max size > 63 μm and total area > 350 μm , type is soft indistinct	1

The early ARM subjects were divided into two groups as close as possible with the AREDS system (Age-Related Eye Disease Study Research Group 2001a) (Table 3.1) and increasing risk for developing late ARM in subjects having drusen and retinal pigment epithelium (RPE) abnormalities (van Leeuwen et al. 2003b; Wang et al. 2003a). Group one included subjects with drusen size greater than 63 μm and group two consisted of subjects with drusen together with focal hyper- or hypopigmentation RPE abnormalities (Table 3.2). Although the AREDS system graded larger drusen with no RPE abnormalities to a higher severity score (LIII), we graded our subjects with distinct or indistinct drusen and RPE abnormalities in the higher risk group (group 2) compared to subjects with only drusen (group 1) (Holz et al. 1994b). This was based upon the findings of van Leeuwen et al. (2003b) who showed a 3 fold increased risk when hard, soft distinct or soft indistinct drusen were seen together with hyper- or hypopigmentation.

Table 3.2. Subject characteristics and grading results

subjects	age	eye	VA	group	grading
1	63	re	6/6	1	LIIa
2	72	re	6/12	1	L IIIb
3	73	re	6/6	2	L IIc (3)
4	72	re	6/12	2	L IIc (3)
5	72	re	6/6 ⁻¹	2	L II c(1)
6	69	re	6/7.5	1	L IIIb
7	71	re	6/12	2	L IIc (3)
8	73	re	6/7.5	1	L IIb
9	77	re	6/7.5	1	L IIIb
10	67	re	6/7.5	2	L IIc (3)
11	73	re	6/9.5 ⁺¹	1	L IIIb
12	77	re	6/9.5	1	L IIIb
13	72	le	6/6 ⁻¹	2	L IIc (3)
14	69	le	6/6 ⁻²	2	L IIc (3)
15	70	le	6/6 ⁻¹	2	L IIc (3)
16	72	re	6/9.5 ⁺¹	2	L IIc (3)
17	68	re	6/9.5 ⁻²	2	L IIc (3)

3.4.3 Cone-mediated mfERGs

The mfERGs (VERIS I, EDI Inc., San Mateo, CA) were recorded monocularly with DTL (Dawson-Trick-Litzkow) thread-electrodes; subjects were optically corrected for the stimulus viewing distance (50 cm). Pupils were maximally dilated with tropicamide 0.5% and phenylephrine 2.5% and the diameters ranged from 8-9 mm for all subjects. The subjects were instructed to watch the centre of a monitor flickering between black and white hexagons. To help to maintain fixation a cross that extended to each corner of the screen was used. The visual stimulus consisted of 103 scaled hexagons displayed on a monitor (width 35.5 deg, height 28 deg) driven at a rate of 67 Hz. The hexagons flickered according to a pseudorandom binary m-sequence ($2^{13}-1$) with a luminance of 100 cd/m² in the white hexagons and 2 cd/m² in

the black hexagons (measured with a Topcon BM-7 luminance colorimeter). Recordings were divided into 16 segments (each about 10 seconds long). Kernel overlap was excluded by running an overlap test before setting up the protocol. Four recording files for averaging were obtained from every subject resulting in a total recording time of about ten minutes per eye (not including resting time between the segments). Retinal signals were band pass filtered (1-300 Hz), sampled every 1.87 ms and amplified (100,000 Grass amplifier). Fixation control was assured by observing the subjects' recordings online on a monitor and by analysing the region of the blind spot after the recordings. Any blinks or small eye movement during the recording segments caused ERG artefacts which were detected online and such segments were rejected and re-recorded.

Before analysing the first order kernel amplitudes and latencies, one spatial averaging (ratio=6) and one noise reduction procedure were performed on the raw mfERG data. Local reduction in the mfERG responses (Martinson 2000; Gerth et al. 2003) as well as reductions by choosing the concentric ring averaging methods have been reported in ARM (Li et al. 2001; Palmowski et al. 2001). We chose two different averaging methods from those previously used for analysing our mfERG results. We adapted one method of Palmowski et al. (2002) who showed that the central averaged amplitude was about two times greater on average than the amplitude of the overall response. A second method was chosen because of reported inferior-superior field asymmetry in the mfERG measures (Parks et al. 1996-1997) as well as in visual fields (Brenton et al. 1986; Katz and Sommer 1986) in subjects with normal vision. Further, we chose the second method because of a suggested rod-cone interaction and that cone survival might be dependent on the rods (Hicks and Sahel

1999; Mohand-Said et al. 2001). Based upon histopathologic evidence that the rod loss and degeneration starts in the lower retina (Curcio 2001) we were interested to see if this was also reflected in reduced cone-mediated function in the superior field. Taking photoreceptor and bipolar cell contribution (Hood et al. 2002) to the a- and b-waveform of the mfERG into consideration, we included the latencies for the first negative peak (N1-latency) and the first positive peak (P1-latency).

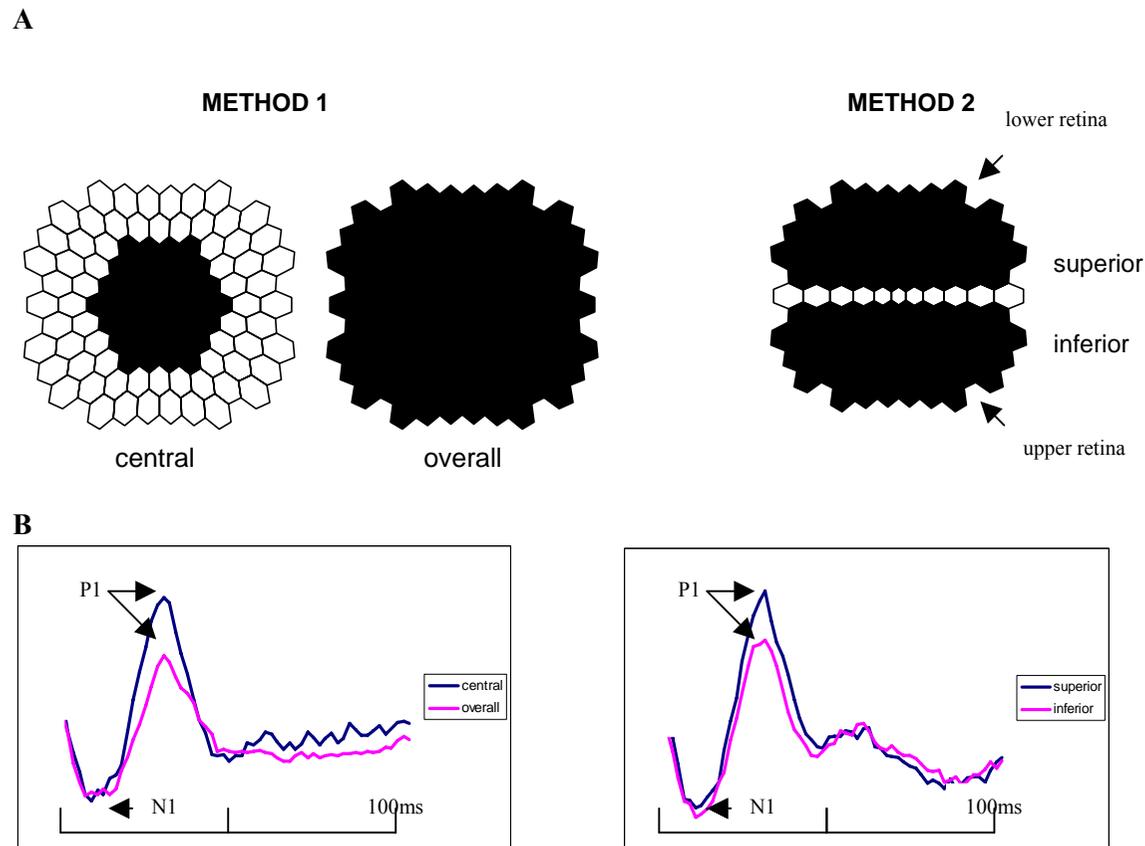


Figure 3.1A,B. **A.** In analysis method 1 we calculated the ratios of the first-order P1-amplitudes, the N1- and P1-latencies between the central 4 rings and the overall response. The analysis method 2 compared the ratios of the first order P1-amplitudes, the N1- and P1-latencies between the inferior and the superior field (without the central horizontal hexagons). **B.** The raw unfiltered first order mfERG responses for method 1 (averaged central and overall responses) and for method 2 (averaged superior and inferior responses) in a control subject showing larger central and superior amplitudes than overall and inferior amplitudes are shown.

In the first method we analysed the ratios between the averaged central four rings and the overall response (method 1) for amplitudes and latencies (Figure 3.1A). In the second method we compared the ratios of the N1P1-amplitude and N1- and P1-latencies between the averaged inferior (upper retina) and superior (lower retina) fields (method 2) (Figure 3.1B). We analysed the first-order kernel responses in our subjects which are thought to reflect the mean local response to all the flashes occurring in a stimulus cycle (Sutter 2000; Keating et al. 2002). On average we found larger amplitudes (mean 1.3 ± 0.2) for the central field and lower amplitudes (mean 0.8 ± 0.2) for the inferior field (upper retina) in our control subjects (Fig 1B). In most of the controls the central N1-latency was faster (mean 0.9 ± 0.1) than the overall response whereas the P1-latency ratios were the same for both methods. In most of the normal controls we found slower N1-latencies (mean 1.1 ± 0.2) for the inferior than for the superior field (Table 3.3).

Table 3.3. The ARM subjects' P1-amplitude and N1-latency ratios of the mfERG results (top half) and mean (AV) psychophysical test results (lower half) compared to those of the control subjects (in brackets) with minimum (Min), maximum (Max), standard deviations (SD) and p-values in comparison to control results.

	<i>P1-amplitude method1</i>	<i>N1-latency method1</i>	<i>P1-latency method1</i>	<i>P1-amplitude method2</i>	<i>N1-latency method2</i>	<i>P1-latency method 2</i>			
AV(norm)	1.3(1.3)	0.99(0.99)	1.04(1.04)	0.85(0.88)	1.0(1.1)	1.1(1.0)			
SD(norm)	0.2(0.2)	0.2(0.1)	0.17(0.11)	0.1(0.2)	0.2(0.2)	0.2(0.3)			
Max(norm)	2(2)	1.3(1.1)	1.64(1.44)	1.1(1.3)	1.5(1.4)	2.2(1.8)			
Min(norm)	1.1(1.2)	0.7(0.8)	0.9(0.9)	0.6(0.6)	0.8(0.8)	0.9(0.9)			
	p=0.5	p=0.8	p=0.9	p=0.6	p=0.6	p=0.8			
*statistically significant									
	<i>age</i>	<i>sat. D-15</i>	<i>desat.D-15</i>	<i>SKILL</i>	<i>Pelli</i>	<i>MS</i>	<i>HC-VA</i>	<i>LC-VA</i>	<i>near VA</i>
AV(norm)	71(68)	1.6(1.1)	2.0(1.5)	42(32)	33(36)	29(30)	0.14(-0.01)	0.48(0.32)	0.24(0.13)
SD(norm)	3.5(5.1)	0.8(0.2)	0.7(0.4)	14(7)	2(3)	1.8(2.5)	0.11(0.13)	0.2(0.17)	0.14(0.12)
Max(norm)	77(75)	3.2(1.6)	3.2(2.4)	73(46)	36(42)	32(34)	0.32(0.3)	0.8(0.8)	0.53(0.45)
Min(norm)	63(58)	1(1)	1.1(1)	22(21)	30(28)	26(22)	0(-0.2)	0.2(0.02)	0.02(-0.02)
		p=0.02	p=0.01*	p=0.01*	p<0.01*	p=0.2	p<0.01*	p<0.01*	p=0.02

3.4.4 Psychophysical and function tests

A number of vision function tests were assessed monocularly; subjects were optically corrected for the relevant distance for each test. All tests were performed using the standardized and recommended procedures. All subjects underwent the following tests: high and low contrast distance visual acuity (HC-, LC-VA) and near visual acuity (near-VA, word reading chart) were measured with the Bailey-Lovie charts scored letter by letter (or word by word) in logMAR units (Bailey and Lovie 1976; Bailey and Lovie 1980; Lovie-Kitchin and Brown 2000). Contrast sensitivity was measured using the Pelli-Robson letter contrast sensitivity chart (Pelli et al. 1988) with letter by letter scoring (Elliott et al. 1990) and low luminance VA was measured using the Smith-Kettlewell Low Luminance (SKILL) card scored by the acuity loss (number of letters) between the light and the dark sides (Haegerstrom-Portnoy et al. 1997). Colour vision was assessed with the Panel D-15 (sat. D-15) and the Lanthony desaturated D-15 (desat. D-15) (Dubois-Poulsen and Lanthony 1973; Lanthony 1978; Committee on Vision 1981; Adams and Rodic 1982; Bowman and Cameron 1984). The error score was expressed as a confusion index (c-index) (Vingrys and King-Smith 1988). A c-index of 1 indicated a correct cap arrangement and errors give larger numbers. Central visual fields (10-2 threshold) were assessed with the Humphrey Field Analyzer (Model 630, Humphrey-Zeiss, San Leandro, CA) and mean sensitivity (MS) was analysed.

3.4.5 Statistical analysis

Based upon reported data in early ARM (Huang et al. 2000; Li et al. 2001; Palmowski et al. 2001) which show a reduction in the central amplitude of about 25% compared to subjects with normal vision we calculated the required sample size

for our study using InStat (GraphPAD, 1.14, 1990, San Diego, CA). To detect a 25% change using a power of 0.8 and α of 0.01 the required sample size was 17.

Results of each test from the ARM group were compared to the control group by independent samples t-tests assuming unequal variances (Microsoft Excel). For Pearson's correlations between the ARM subjects' results, data were analysed with the Statistical Package for the Social Sciences-SPSS-11. For correlations a probability level of $p \leq 0.05$ was considered statistically significant. Because of the number of statistical comparisons made, a stricter probability level of $p \leq 0.01$ was adopted for statistical significance for all other analyses.

3.5 RESULTS

We found significantly reduced vision as measured by the psychophysical tests and not significantly reduced results for the mfERG responses of the ARM group compared to the age-matched control group (Table 3.3, Figure 3.2A-D). The bold numbers in brackets in Table 3.2 show the mean results of the normal control group and the bold numbers without brackets show the significant p-values for the ARM group results compared with the normal age-matched data. In Figure 3.2A-D the first order mfERG waveforms of each of the ARM subjects for the averaged central, overall, inferior and superior fields compared to the mean of the 20 control subjects (bold line) are shown.

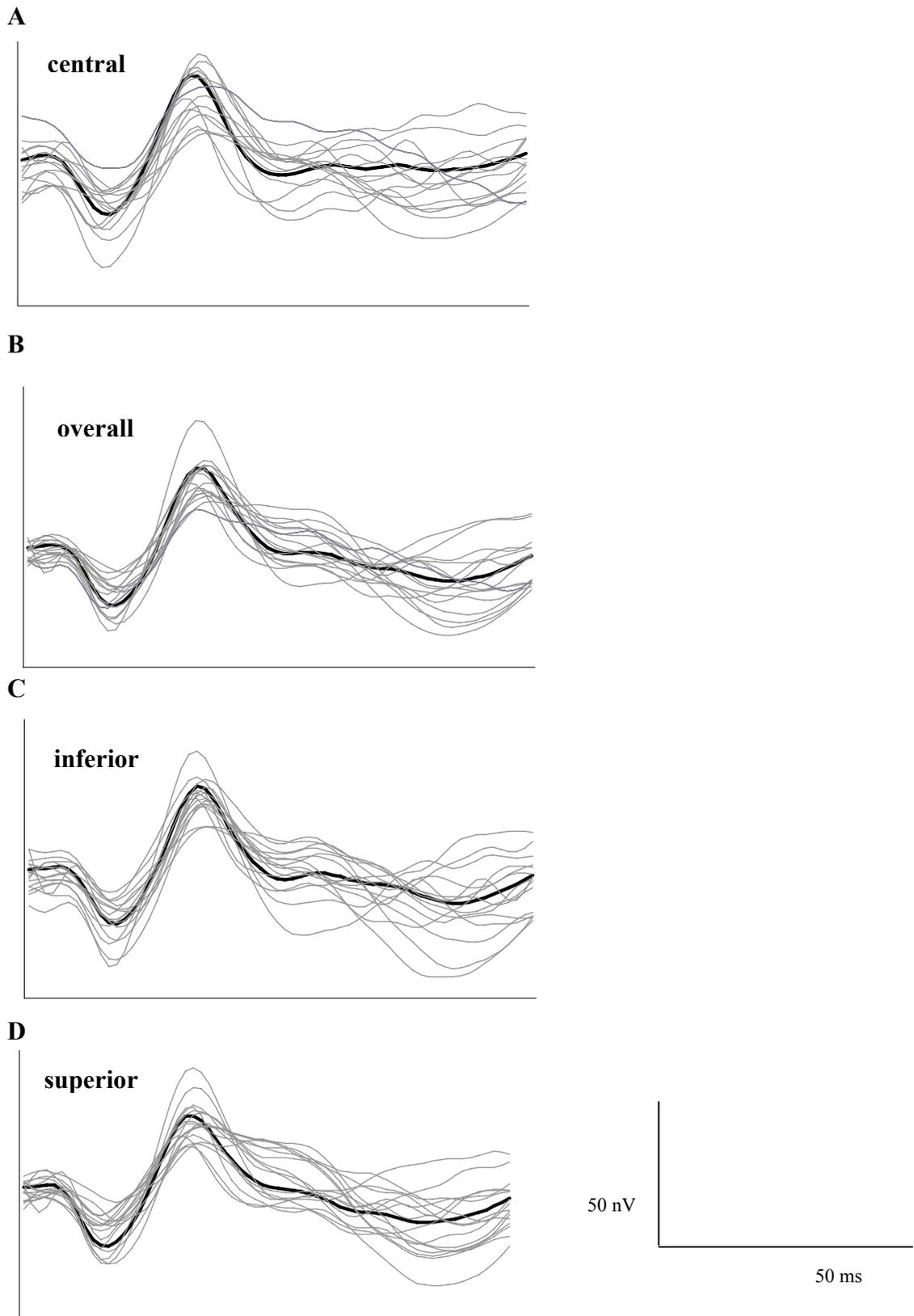


Fig 3.2A-D. The first-order mfERG responses of the averaged central (A), the overall (B), the inferior (C) and superior (D) fields of each of the ARM subjects (thin lines) and the mean central, overall, superior and inferior mfERG first order kernel responses (bold) of the control subjects are shown.

3.5.1 Psychophysical tests and mfERG in early ARM subjects

Psychophysical results were analysed for 20 control eyes and 17 ARM eyes, except for one ARM subject who was not able to perform the desaturated colour vision test. As shown in Table 3.3 (lower half), the vision results from the desaturated Panel D-15 colour vision test, the SKILL charts, Pelli-Robson contrast sensitivity and HC- and LC-VA were significantly worse ($p \leq 0.01$) for the ARM group compared to those for the control group. The desaturated Panel D-15 revealed arrangement errors with a tritan axis in eleven of our subjects. Two subjects demonstrated substantial arrangement errors indicating no specific deficiency, three showed normal results and one subject could not perform the test.

The cone-mediated mfERG was performed in 17 eyes (14 right and 3 left eyes) of the ARM group and in one eye each (14 right and 6 left eyes) of our control subjects ($n=20$). Table 3.3 (top half) shows the mean N1P1-amplitude and N1- and P1-latency ratios for all ARM and control subjects for both methods.

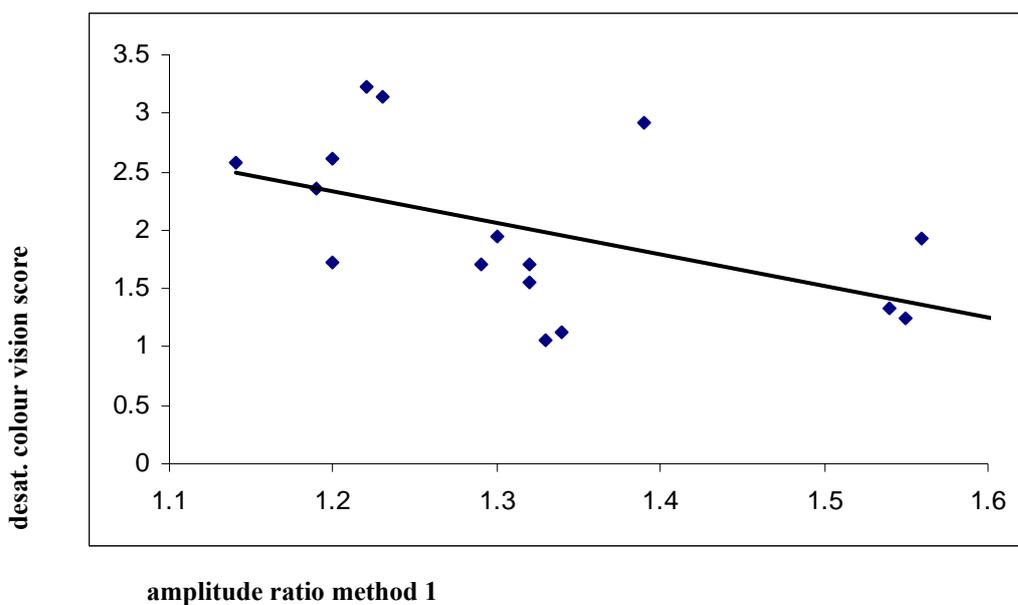


Figure 3.3. Lower central amplitudes (lower ratios) were correlated with poorer colour vision (higher scores).

Statistically significant correlations were found between the mfERG amplitude ratios for both methods and the desaturated colour vision test. Thus, lower central and inferior field (upper retina) amplitudes were associated with poorer desaturated colour vision ($r = -0.5$, $p \leq 0.05$) (Figs. 3.3 and 3.4). No significant correlations were found between the mfERG latency ratios and the psychophysical measures nor was there a significant correlation between mfERG variables and the morphological fundus changes.

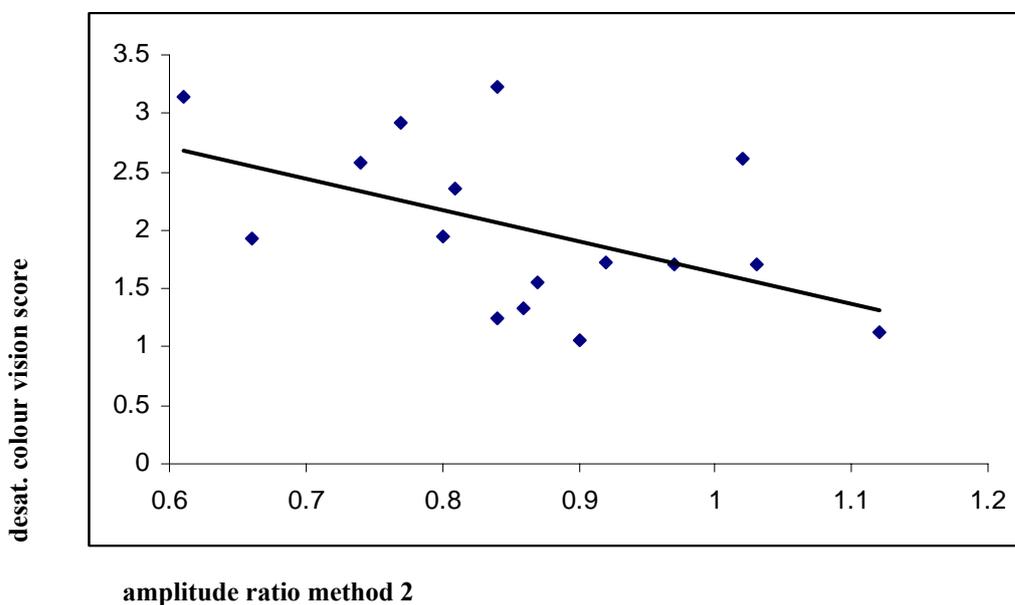


Figure 3.4. Lower amplitudes (lower ratios) in the inferior field (upper retina) were correlated with poorer colour vision (higher scores).

3.6 DISCUSSION

This study was conducted to investigate the relationships between psychophysical vision function and objective retinal function in subjects with early ARM but relatively good high contrast visual acuity. We found a significant correlation between the averaged first-order mfERG results and colour vision showing lower central and upper retinal mean amplitudes with poorer colour vision outcomes. However, fundus changes were not correlated with the first order mfERG responses

and the mfERG could not discriminate between the control and the early ARM group. To some extent these correlation results are in agreement with the recent findings of Gerth et al. (2003) who found few significant morphologic functional correlations in their early ARM subjects. They examined averaged (central 15° versus peripheral 15°) response densities and implicit times and showed poor correlations, but found a better correlation by using the scalar product method which is very sensitive to changes in the waveform shape (Sutter and Tran 1992; Bearse and Sutter 1996). Our prediction of lower cone-mediated responses in the superior field (lower retina) because of a suggested rod triggered cone degeneration (Hicks and Sahel 1999; Mohand-Said et al. 2001) could not be verified.

Most of our ARM subjects did not report any visual complaint. Although we found significantly reduced HC- and LC-VA, contrast sensitivity and desaturated colour in the ARM group compared to the control group the mfERG measures were within the normal range. Significantly reduced mfERG results (Li et al. 2001; Palmowski et al. 2001; Gerth et al. 2003) in early ARM have been shown in other studies with various sample sizes and visual acuities better than 6/10 (Huang et al. 2000; Li et al. 2001; Palmowski et al. 2001). By choosing concentric averaging methods Li et al. (2001) reported reduced amplitudes and delayed latencies in their 15 early ARM eyes. Huang et al. (2000) and Palmowski et al. (2001) found a decrease of the concentric ring averaged amplitudes and latencies in eight and ten subjects, respectively. Gerth et al. (2003) investigated the local first order kernel responses in 31 eyes of 20 ARMs with visual acuities better than 6/15 and detected impaired mfERG responses even in more peripheral, funduscopically normal areas. One reason for the differences between these studies and ours may be the comparison between different

age-groups. The control subjects of Palmowski et al. (2001) and Huang et al. (2000) were younger (mean 60.9 and 57.7 years, respectively) than their ARM subjects (mean age 66.7 and 66.5 years, respectively). We know that there is an age effect on the mfERG from previous studies (Nabeshima et al. 2002; Seiple et al. 2003). Seiple et al. (2003) showed a progressive amplitude decrease of 10.5% per decade for the P1-amplitude but less change for the P1-latencies (<2%) per decade. It might be possible that other studies measured an age-effect in their ARM subjects rather than reduced results due to the disease itself. Given that our subjects were age-matched within a very small range (most of the control and ARM subjects were between 67 and 72 years), this could have influenced our non-significant results between the early ARM and control group. Further there is still controversy about which parameter, amplitude or latency, is more sensitive in early ARM (Li et al. 2001; Palmowski et al. 2001). This inconsistency between studies might also reflect the nature and varying results of early disease. A significant outcome for our mfERG results from this particular study compared to an age-matched control group might have been difficult to achieve because of the restricted range of deficiencies in the early ARM subjects we investigated. This could have been also due to our subject source; our subjects were mainly selected from the database of an optometry clinic and were undergoing routine examination rather than presenting because of symptoms of vision loss.

The lack of significant differences in mfERG results between the ARM and control groups may also be related to our test system. It is possible that the luminance of our mfERG display (100 cd/m²) together with the use of DTL electrodes lead to an increased signal/noise ratio. However, our screen luminance is within the range of

the ISCEV recommendation (screen luminance 100-200 cd/m²) (Marmor et al. 2003) and there are studies using relatively low luminance conditions in various diseases that have shown reliable results (Chan and Brown 2000; Kretschmann et al. 2000). Chan and Brown (2000) investigated glaucoma suspects with ocular hypertension in eight subjects using a mean luminance of 61 cd/m² (and DTL electrodes) and showed separation of the control and ocular hypertension groups. Kretschmann et al. (2000) reported reliable mfERG results in various diseases of the retina including maculopathies by using DTL electrodes and a mean luminance of 51.8 cd/m². Although low luminance may lead to smaller responses, Kretschmann et al. (2000) suggested that longer recordings can be performed to improve the quality as choosing higher luminance might induce more stray light. We obtained four recording files for averaging to improve signal/noise ratio for each subject.

Previous studies in which a number of psychophysical tests were performed have shown functional deficits in early ARM (Eisner et al. 1991; Midena et al. 1997). Midena et al. (1997) found macular recovery function, the central visual field and spatiotemporal contrast sensitivity to be the most reliable indicators of impairment. Eisner et al. (1991) correlated the fundus appearance in early ARM with subjective function tests and found a reduced S-cone mediated sensitivity and slower rates of dark adaptation in their high risk eyes (based on larger drusen size). They demonstrated that a D-15 failure was specific for their high risk eyes. Conversely Midena et al. (1997) could not show a significant difference in colour vision using the Farnsworth-Munsell FM 100 hue test in their ARM subjects compared to a control group. They explained this by the character of the disease and the inadequacy of commercial tests to detect subtle defects. Vingrys et al. (1992) showed that the

FM 100-hue test failed to diagnose the type of defect in 21% of 95 congenitally colour deficient males, and that it had a higher rate of misdiagnosis (13%) than a combination of the Panel D-15 and the Lanthony desaturated D-15 (7%) tests. We found a tritan defect in most of our ARM subjects, which is consistent with the results of other studies investigating the S-cone pathway in the early course of ARM (Collins 1986; Eisner et al. 1991).

The cellular origins of the mfERG signal are under investigation, as it reflects the sum of the contribution from various retinal cells. Hood et al. (1997) showed that there was a strong correlation between the a- and b-wave of the full-field ERG and the mfERG by slowing down the stimulation sequence. In further animal studies Hood et al. (2002) developed a model of the mfERG origins by pharmacologically blocking particular cells and circuits in the retina. They found ON and OFF-bipolars mainly contributing to the shape of the first order kernel a- and b- waveform response and only a small contribution of the photoreceptors in the descending part of the a-wave. Few studies have correlated objective mfERG function with psychophysical measurements in an attempt to identify common mechanisms between objective and subjective tests (Holopigian et al. 2001; Holopigian et al. 2002; Seiple et al. 2002). Seiple et al. (2002) compared visual field and mfERG thresholds in normal eyes. They matched the stimulus parameters as far as possible to investigate how methodological differences contribute to the relationships between local psychophysical and mfERG responses. They still found higher values for the mfERG thresholds than those for the central visual field suggesting that these two tests measure different aspects of vision. Given that visual fields are typically performed at lower adaptation levels than the mfERG they also suggested that their

relationship depends on how a retinal disease affects sensitivity as a function of adaptation level. The significant correlations between the mfERG and the desaturated colour vision test might signify similar cone-mediated cell mechanisms in the early course of the disease. Both tests are proven to be sensitive in detecting early changes in cone function (Lanthony 1978; Bowman and Cameron 1984; Chappelow and Marmor 2000).

Early cone-mediated impairment despite good visual acuity, in highly sensitive psychophysical tests of flicker sensitivity and spatial and temporal contrast sensitivity have been previously reported in subjects with early ARM (Falsini et al. 2003; Phipps et al. 2003). Falsini et al (2003) investigated 19 subjects and showed a loss of the cone-mediated flicker sensitivity by using focal ERGs. They suggested early degenerative changes of the cone photoreceptors and a possible reduced quantum catch despite normal cone numbers. This is supported by the histopathological findings of Curcio et al. (1996) of misshapen parafoveal cones but a normal foveal mosaic in early ARM. Further, Phipps et al. (2003) showed reduced cone-mediated kinetic and steady state thresholds to chromatic and achromatic stimuli presented on a TV monitor. They showed the necessity of a combination of kinetic and steady state testing to identify visual deficits in early ARM as those tests assay different aspects of visual function.

There was a greater functional impairment detected by the psychophysical tests than by the averaged first-order kernel mfERG test in the ARM subjects compared to the controls. This might be due to differing sensitivity and adaptation properties of the cells in the inner and outer retinal layers in the early course of ARM. Analysis of

higher-order kernel mfERG responses which are thought to reflect fast adaptive mechanisms of the inner retina has been shown to be sensitive in diabetes with the conventional mfERG paradigms (Palmowski et al. 1997). However, other mfERG protocols reflecting adaptive properties and inner retinal function have been suggested (Sutter and Bearse 1998; Sutter et al. 1999). Recently Shimada et al. (2001) introduced a new mfERG protocol in subjects with diabetes without diabetic retinopathy. They suggested that by modulating the m-sequence using different multifocal stimuli and inserting global flashes the new paradigms were more sensitive to detecting early changes. They found larger than normal reductions in amplitudes of the focal responses produced by the global flash in their subjects without retinopathy.

Applying new stimulation protocols with the mfERG in early ARM subjects might be helpful in detecting functional impairment before psychophysical measures are impaired. Although the use of more hexagons (e.g 241) would probably also result in a better detection of early changes due to higher spatial resolution, this would also give longer recording times and a poorer signal/noise ratio (Poloschek and Sutter 2002). Especially in untrained subjects such a protocol is not practical for clinical use. A number of studies (Curcio et al. 1996; Owsley et al. 2000; Scholl et al. 2004) have shown that rods are earlier affected than cones in early ARM which might be another approach in detecting early ARM.

Our study demonstrates that desaturated colour vision is significantly related to the mfERG suggesting that the first order responses operate at a selective and sensitive level. The standard cone-mediated mfERG did not discriminate between the groups

and was not correlated to the fundus morphology in our sample, so that further work on these issues might be needed or using different mfERG protocols might be of greater value.

3.7 Acknowledgements

The authors thank Dr. Lawrence Lee for his assistance in recruiting subjects.

3.8 REFERENCES (see Master Reference List)

CHAPTER 4

Cone- and rod-mediated multifocal

electroretinogram in early age-related maculopathy

4.1 ABSTRACT

4.1.1 Purpose. To investigate the cone- and rod-mediated multifocal electroretinograms (mfERG) in early age-related maculopathy (early ARM).

4.1.2 Methods and subjects. We investigated the cone and rod-mediated mfERG in 17 eyes of 17 subjects with early ARM and 16 eyes of 16 age-matched control subjects with normal fundi. All subjects had a visual acuity of 6/12 or better. We divided the ARM subjects into two groups based on drusen size and retinal pigment epithelium abnormalities - a less advanced (ARM1) and a more advanced (ARM2) group. The mfERG data were compared to templates derived from the control group. We analysed the mfERG results for the central and peripheral fields (CP method) and the superior and inferior fields (SI method).

4.1.3 Results. While the mean cone results showed no statistically significant difference between the groups, the rods showed significantly delayed responses in the ARM1 group for the CP and the SI methods, but not in the ARM2 group, although there was a trend of longer latencies compared to the control group.

4.1.4 Conclusion. Our results show a functional impairment of the rods in early ARM subjects. Because there is histopathological evidence showing earlier rod than

cone impairment in early ARM, following the rod function with the mfERG might be helpful in diagnosis or for monitoring the progression of early ARM.

4.2. KEYWORDS

Multifocal electroretinogram, mfERG, cones, rods, early age-related maculopathy, early ARM

4.3 INTRODUCTION

Recent histopathological studies by Curcio and colleagues (1993; 1996; 2000; 2001) have shown a preferential vulnerability of rods compared to cones in ARM eyes. They found no difference between normal age-matched eyes and early ARM eyes in the foveal mosaic, but parafoveally there was a localized rod loss together with misshapen cone inner segments in early ARM eyes. Furthermore, they demonstrated that the rod loss began in the inferior retina during normal aging. Earlier histopathologic studies in aging eyes also indicated preferential rod damage with elongation and nuclear displacement starting at age 40 (Marshall et al. 1979; Gartner and Henkind 1981). Holopigian et al. (1997b) investigated the peripheral cone and rod function in subjects with early ARM by obtaining dark-adaptation curves, electro-oculograms (EOG) and full-field electroretinograms (ERG). They found no difference for the cones compared to age-matched controls but abnormal absolute thresholds and cone-rod break times as well as impaired electroretinographic measures in the fullfield ERG for the rod system. Owsley and colleagues (2000; 2001) measured scotopic sensitivity at 52 loci in the central 38 degree field and showed delayed rod-mediated dark adaptation in early ARM. Sunness and Massof (1986) tried to differentiate between ARM stages by performing focal EOGs but

found that the focal EOG was not sensitive enough to discriminate between ARM subgroups. Recently Scholl et al. (2004) showed normal or mildly abnormal photopic sensitivity, but moderately to severely reduced scotopic sensitivity in ARM subjects with central increased fundus autofluorescence corresponding to foveal drusen. They performed fine matrix mapping with a modified Humphrey field analyser depending on the location of the area of increased fundus autofluorescence imaged with a confocal scanning laser ophthalmoscope. Curcio et al. (2000) suggested that tests of rod function might be advantageous, and might permit detection of ARM at earlier stages than do standard tests of cone function.

The multifocal electroretinogram (mfERG) developed by Sutter and Tran (1992) can give valuable information about the functional condition of the central retina (Palmowski et al. 1997; Kretschmann et al. 1998c; Seeliger et al. 1998a; Palmowski et al. 2001; Palmowski et al. 2002). Most previous studies have been performed under photopic conditions, which are thought to reflect mainly cone-mediated photoreceptor and bipolar function (Hood et al. 1997; Hood et al. 2002). Although reduced amplitudes and delayed latency of the cone mfERG have been demonstrated with age (Gerth et al. 2002; Jackson et al. 2002a; Seiple et al. 2003) and in different stages of ARM (Huang et al. 2000; Jurklies et al. 2000; Martinson et al. 2000; Li et al. 2001) mainly with concentric ring averaging methods, there have been no studies identifying the functional impairment of rods. Recently Hood et al. (1998b) developed a mfERG protocol for isolating rod responses in humans and Holopigian and colleagues (2001; 2002) demonstrated the clinical use of this protocol in retinitis pigmentosa and progressive cone dystrophy.

Based on these histological and psychophysical results, our main goal was to measure the function of both cones and rods in different advanced ARM groups, using the mfERG and a curve fitting method for objectively characterising mfERG parameters.

4.4 MATERIALS AND METHODS

4.4.1 Subjects

Thirty-three subjects were selected from the Optometry Clinic at the Queensland University of Technology, Brisbane or were referred by local ophthalmologists. Seventeen subjects made up the early ARM group (9 female, 8 male, age range 60-75 years, mean age 70 years) and 16 subjects comprised the age-matched control group (8 females and 8 males, age range 58-72 years, mean age 68 years). All subjects had distance visual acuity of 6/12 or better. They underwent a full clinical examination, including slitlamp and fundus photography and if necessary fluorescein angiography to exclude choroidal neovascularisation. All subjects who were enrolled in the study gave written informed consent and the tenets of the Declaration of Helsinki and the requirements of the University Human Research Ethics Committee of the Queensland University of Technology were followed.

Slitlamp photographs for grading the lens were taken and subjects with posterior subcapsular cataract and cortical or nuclear opacities higher than grade one according to the templates of the Age-Related Eye Disease Study (AREDS) clinical lens standards were excluded (Age-Related Eye Disease Study Research Group 2001b). Colour fundus photographs (Zeiss Jena Mydriatic Fundus Camera) of the central 30 degrees of the posterior pole (centred on the fovea) were taken and retinal changes

were graded independently by two experienced observers (BF, PS) by using a set of the Wisconsin age-related maculopathy standards with example photographs and a grid with grading circles (AREDS Reading Center, University of Wisconsin, Madison) (Age-Related Eye Disease Study Research Group 2001a). One observer (PS) was masked to the subjects' functional results and agreement was achieved in all subjects with judgments of the other investigator (BF, not masked).

Table 4.1. ARM subjects divided into two groups (ARM1 and ARM2) according to the AREDS ARM main levels.

	groups
<u>Drusen size μm (LI)</u> <63 μm and total area <125 μm	ARM 1
<u>presence of one or more (LII)</u>	
a. Drusen max size $\geq 63\mu\text{m}$ but < 125 μm	
b. Drusen total area $\geq 125\mu\text{m}$	
c. Retinal pigment epithelial abnormalities consistent with AMD, defined as one or more of the following in the central or inner subfield	
1. Depigmentation present	
2. Increased pigment $\geq 125\mu\text{m}$	
3. Increased pigment present and depigmentation at least questionable	
<u>presence of one of the following (LIII)</u>	ARM 2
a. Drusen max size $\geq 125\mu\text{m}$	
b. Drusen max size $\geq 63\mu\text{m}$ and total area > 372 μm , type is soft indistinct	
c. Drusen max size $\geq 63\mu\text{m}$ and > total area 650 μm and type is soft distinct	
d. geographic atrophy within grid but none at the centre of macula	

We defined early ARM as the presence of either hard or soft distinct drusen less than 125 μm in size with or without retinal pigment epithelial (RPE) abnormalities or soft indistinct drusen larger than 125 μm . Both types of drusen have been shown to damage cone and rod inner and outer segments (Johnson et al. 2003). We divided our subjects into two groups (ARM1 and ARM2) according to the AREDS AMD levels

(Age-Related Eye Disease Study Research Group 2001a) (ARM1= AREDS ARM level 1 and level 2; ARM2= AREDS ARM level IIIa-c) (Table 4.1). The ARM1 group (less advanced) consisted of 11 eyes with drusen size less than 125 μm and RPE abnormalities and the ARM2 group (more advanced) included six eyes with indistinct drusen greater than 125 μm and/or a total area 372 μm determined “by mentally moving all drusen together” (Age-Related Eye Disease Study Research Group 2001a) and comparing this area with those of the standard circles of the Wisconsin grading system. These two groups represent an increasing risk to develop late ARM (Wang et al. 2003a). The control subjects (N group), with visual acuities of 6/6 or better and normal fundi, made up the third group (16 eyes). Table 4.2 shows the visual acuities and grading results for each of the ARM subjects.

Table 4.2. The grading result for each subjects’ right (re) or left eye (le) and the distance visual acuity (VA).

subjects	eye	VA	group
a	re	6/6	ARM1
b	re	6/12	ARM2
c	re	6/6	ARM1
d	re	6/12	ARM1
e	re	6/6 ⁻¹	ARM1
f	re	6/7.5	ARM2
g	re	6/12	ARM1
h	re	6/7.5	ARM1
i	re	6/7.5	ARM2
j	re	6/7.5	ARM1
k	re	6/9.5 ⁺¹	ARM2
l	re	6/9.5	ARM2
m	le	6/6 ⁻¹	ARM2
n	le	6/6 ⁻²	ARM1
o	le	6/6 ⁻¹	ARM1
p	re	6/9.5 ⁺¹	ARM1
q	re	6/9.5 ⁻²	ARM1

4.4.2 mfERGs

The mfERGs (VERIS I, EDI Inc, San Mateo, CA) were recorded monocularly using DTL thread-electrodes and optical correction for the stimulus viewing distance (50 cm). Pupils were maximally dilated with tropicamide 0.5 % and phenylephrine 2.5 %. The subjects were instructed to watch the centre of a monitor flickering between black and white hexagons. To help to maintain fixation a cross extending to each corner of the screen was used. The stimulus for the cones was a 103 hexagon array (35.5 x 28 deg) and for the rods was a 61 hexagon array (33 x 28 deg). Retinal signals were bandpass filtered (1-300 Hz) and amplified (Grass P5 amplifier, x 100000); blinks or small eye movements causing ERG artifacts during the recording segments were detected online and those segments were rejected and re-recorded. Fixation was controlled by observing the subjects' recordings online on a monitor and by analysing the region of the blind spot after the recordings. An overlap test for both protocols was performed to exclude kernel overlap.

4.4.2.1 Cone-mediated mfERG:

The frame rate of the 103 hexagon display was 67 Hz, and the hexagons flickered according to a pseudorandom binary m-sequence ($2^{13}-1$ steps in length), with a luminance of 100 cd/m² for the white hexagons and 2 cd/m² for the black hexagons (measured with a Topcon BM-7 luminance-colorimeter). Recordings were divided into 16 segments (each about 11 seconds). Four files for averaging were obtained from every subject, resulting in a total recording time of about 10 minutes per eye (not including resting time between the segments). Figure 4.1A shows the hexagonal arrays for the cones with the trace arrays of a control subject below.

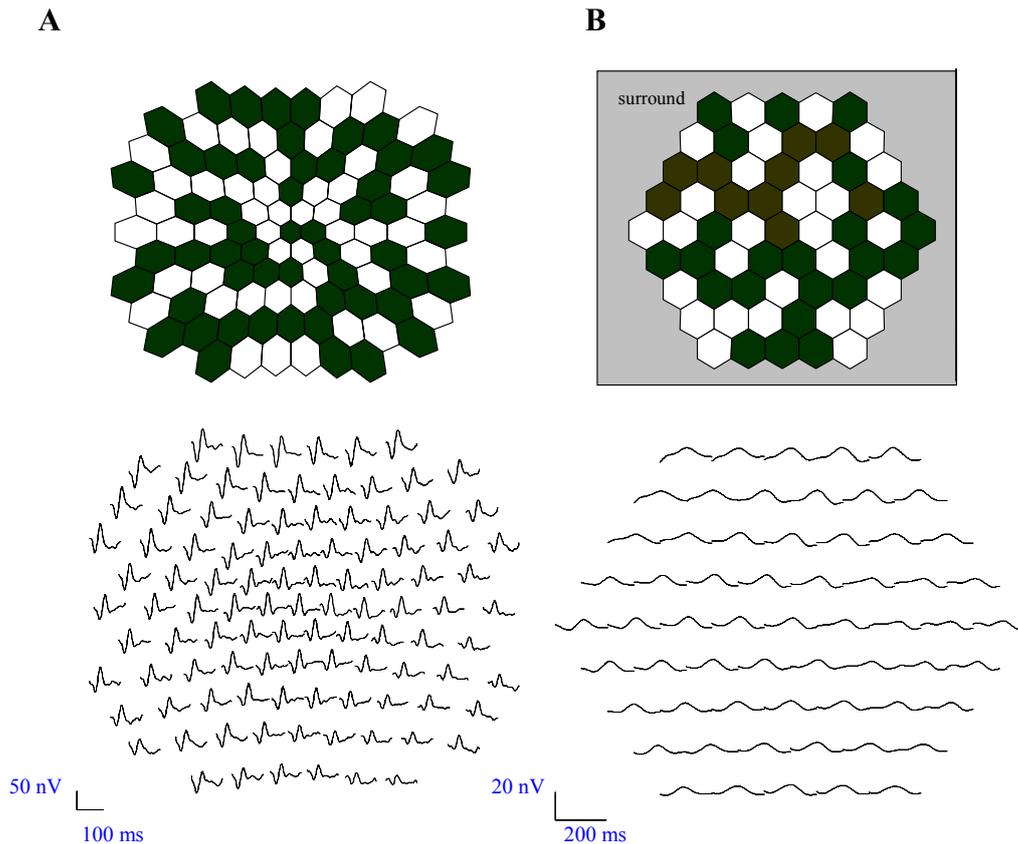


Figure 4.1A, B. The mfERG stimulus array (top) with (A) 103 scaled hexagons for the cones (B) 61 unscaled hexagons and a surround for the rods and the filtered normal responses from a trained control subject (lower panel) right eye. Note the different scaling for the rod-mediated traces and the lower responses nasally indicating the region of the blind spot.

4.4.2.2 Rod-mediated mfERG:

Rod-mediated mfERGs were recorded by applying a method similar to that of Hood et al. (1998b). We used a blue Wratten 47B filter (W47B) and slowed the stimulus sequence by inserting 3 blank frames between stimulus frames. We performed control experiments to find the optimal stimulus luminance and surround conditions to minimize stray light influence (Hood et al. 1998b). We performed several stimulus luminance experiments by varying the neutral density (ND) filters (W47B alone, W47B+ 1.0 ND, and W47B+ 1.5 ND) with no added surround and maximal ND filter set-up (W47B+ 1.5 ND) with surround. Figure 4.2 shows the summed overall unfiltered rod responses to different surround luminances (0.012 cd/m^2 , 0.009 cd/m^2 ,

0.004 cd/m²) demonstrating decreased amplitudes with increased surround luminance. With no surround (0.0005 cd/m²) a large stray light response was found.

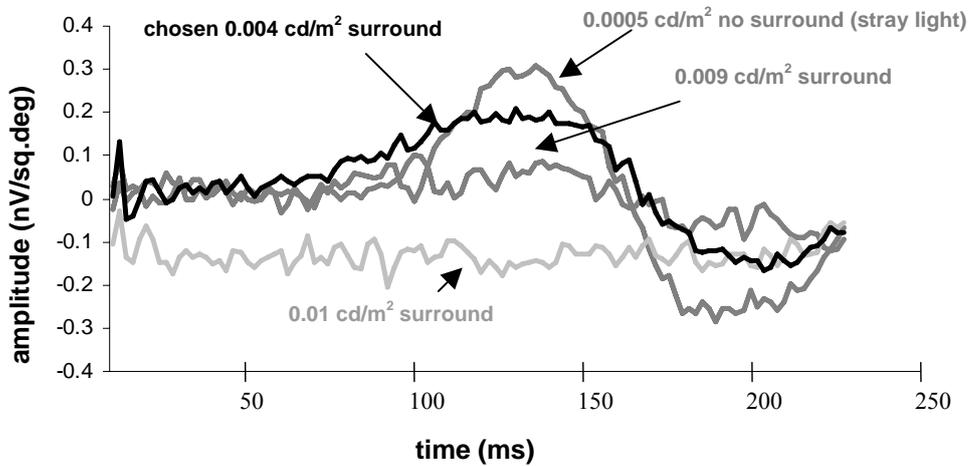


Figure 4.2. The unfiltered rod-mediated mfERG overall response for a trained subject for different surround conditions (no surround=0.0005 cd/m², 0.004 cd/m², 0.009 cd/m², 0.01 cd/m²). A large stray light response was found without a surround and an extinguished response with a bright surround (0.01 cd/m²) was evident. Note that the raw overall amplitude values are shown in nV/sq.deg. (absolute amplitude divided by the area from which the response was evoked).

For our protocol we chose the lowest light condition and the blue filter (W47B+1.5 ND, 0.004 cd/m² surround) which gave us the highest rod response. Thus, the luminance levels for our rod protocol were 0.0098 cd/m² for the bright, 0.0005 cd/m² for the dim hexagons and 0.004 cd/m² for the surround (calculated from measurements made with a Topcon BM-7 luminance-colorimeter).

After 40 minutes of dark adaptation, the rod mfERGs were recorded with a stimulus array of 61 equally sized hexagons which flickered concurrently according to a pseudorandom binary m-sequence (2¹³-1 steps in length). One recording was obtained for each subject; it was divided into 32 segments (each lasting about 21 sec) resulting in a total recording time of about 11 minutes (not including resting time between the segments). Figure 4.1B shows the hexagonal array for the rods and a

trace array of a young and trained control observer. A different scaling for the rod traces was chosen to point out the different and smaller waveforms compared to the cones. The rod-mediated waveforms are much smaller and broader and show a later, less distinct peak compared to the cone-mediated response (Hood et al. 1998b). Although local (foveal and optical disc) responses are difficult to obtain (Hood et al. 1998b) the reduced nasal responses in Figure 4.1B possibly indicate the blind spot area. As with the cone-mediated mfERG (Keating et al. 2000) the blind spot area was not evident in every subject and a response could be obtained in this area due to hexagon size and overlap of the stimulus elements.

4.4.2.3 Analysis

We examined the first order kernel ERG response which is the mean local response to all the flashes occurring in a stimulus cycle (Sutter 2000; Keating et al. 2002). Currently there are three methods available for analysing the mfERG data. The conventional peak to peak (N1P1) amplitude and implicit time (N1-latency, P1-latency) method, the scalar product method (Sutter and Tran 1992; Bearse and Sutter 1996) and a newer method using a least-squares fitting procedure developed by Hood and Li (1997). We applied Hood and Li's (1997) method which has proven to be valuable in hereditary retinal diseases, diabetes and in establishing normative data for the mfERG (Fortune et al. 1999; Holopigian et al. 2001; Holopigian et al. 2002; Seiple et al. 2003). We chose this curve fitting procedure (Matlab, Mathworks, Natick, MA) to detect slight functional deficits in our early ARM subjects who had relatively good visual acuity. Further, we thought the conventional peak to peak amplitude and implicit time method to be less accurate for the rod results as the rod functions show no distinct peak and are broad, small and low frequency potentials

(Fig. 4.1B) (Hood et al. 1998b). We derived normal templates for each of the 103 and 61 hexagons from the control group. We then fitted each ARM subjects' results to those by varying two parameters. One parameter scaled the amplitude (a-scale) at each point in the hexagon array to minimize the difference between the response and the template values and the other parameter was a multiplicative scaling of time (t-scale) that stretches the entire waveform. For each hexagon, a least-squares fitting procedure was used to find the best fit. For each record two values were available: an amplitude (a-scale) and a latency (t-scale) value which indicated values relative to the normal template. In addition, an error statistic (statfit) described the quality of the fit to which both the a-scale and the t-scale contributed. A perfect fit or statfit value of 0.0 is the result of an a-scale and t-scale value of 1.0 which indicates that the values are equal to the mean of a control group. For example an a-scale of 0.8 indicates that the response is 80% that of the mean control group value. Higher t-scale values such as 1.2 indicate 20% longer latencies than the mean from the control template (Hood and Li 1997).

Before fitting the cone and rod responses, the data were spatially averaged once (ratio=6) and the VERIS noise reduction procedure (NRP) was performed (Sutter and Tran 1992). Signals were lowpass filtered at 80 Hz (cones) and 15 Hz (rods) resulting in better measurable signals for the fitting method without appreciable amplitude loss; cone and rod data were fitted over 45 ms (from 15 ms to 60 ms) and 50 ms (from 60 ms to 110 ms) respectively, to avoid early and late transients.

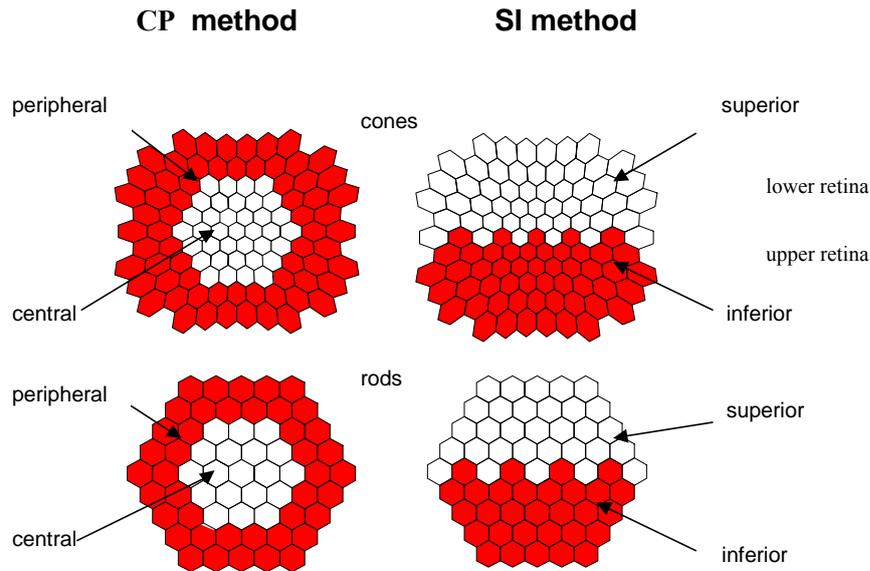


Figure 4.3. In the CP method we analysed the mean results from the central hexagon and the surrounding 3 rings for the cones and the central hexagon and the surrounding 2 rings for the rods and compared those with the means from the outer peripheral rings. The SI method compared the mean results from the superior (lower retina) and inferior (upper retina) fields.

To determine which parameter (a-scale or t-scale value) best describes early ARM we analysed the results by averaging the a-scale and t-scale results for the central (acen, tcent) and the peripheral fields (aperi, tperi), (CP method) and the superior (asup, tsup) and the inferior fields (ainf, tinf), (SI method) which correspond to the lower and upper retina respectively in our normal and ARM subjects (Fig. 4.3).

We chose the CP and SI methods for the cones and the rods because of histopathological evidence of preferential rod vulnerability beginning in the lower retina and parafoveally during aging and in early ARM, and a hypothesized cone-rod interaction previously suggested by Hicks and Sahel (1999). The reason for not excluding the central hexagon, which is supposed to be a rod-free area was the relatively large hexagon size we chose (about 3 degrees) which probably included some rod responses. Additionally Hood et al. (1998b) discussed the difficulty of obtaining local rod responses even with optimal recordings and found it hard to

discern the fovea and the optic disc. Choosing smaller hexagons for more precise local rod distributions (Hood et al. 1998b) would have resulted in a poorer signal-to-noise ratio and would have required longer recording sessions, which in our older subjects we thought was too demanding.

The mean statfit value for cones (0.3 ± 0.1 for all fields) and for the rods (0.3 ± 0.1 for the central and inferior field and 0.4 ± 0.1 for the peripheral and superior fields) showed low values indicating that the template fits were accurate over the whole field.

Cone-mediated mfERGs were recorded from 17 ARM eyes and from 16 control eyes. Rod-mediated mfERGs were recorded from 14 ARM eyes and 13 control eyes (ARM subjects l, p, q and three control subjects could not perform the rod mfERG procedure). Recent studies suggested using a quality (goodness) of the fit (Hood and Li 1997; Holopigian et al. 2001; Holopigian et al. 2002) criterion for deciding which records are responses and which are noise. After examining our data we set a statfit value of lower than 0.8 to identify a response from noise (Fig. 4.4). Results higher than 0.8 were considered as noise. The records of the left eyes were mirror-imaged so that appropriate parts of the retina were being compared across eyes.

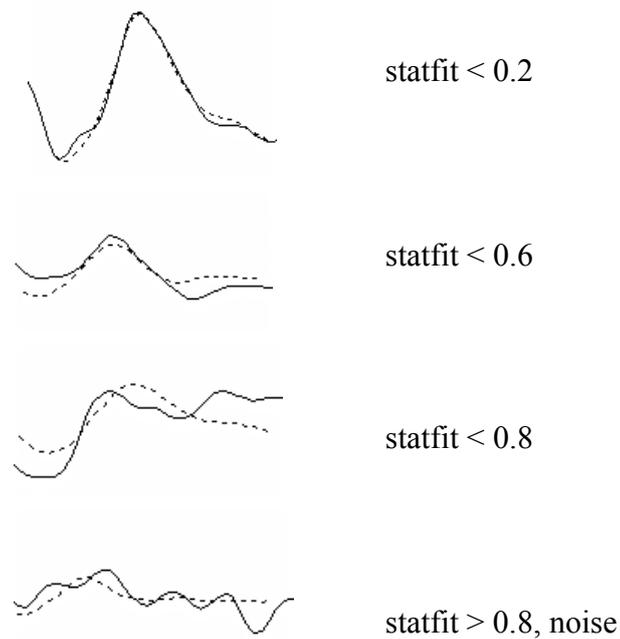


Figure 4.4. The ARM subjects' local responses (solid lines) were fitted against templates for each location (dashed lines) derived from the normal age-matched controls. A goodness of fit cut off of 0.8 was chosen. Statfit values greater than 0.8 were considered as noise and discarded.

4.4.3 Statistical analysis

Data were analysed with the Statistical Package for the Social Sciences (SPSS-11). Repeated measures analysis of variance (ANOVA) and posthoc tests (LSD) were conducted to compare the effect of retinal location (CP and SI methods) and subject groups and to investigate if the groups had different effects at the different retinal locations.

4.4.4 Repeatability of rod-mediated mfERG data

To show the reliability of the rod-mediated mfERG responses we compared the local amplitudes and latencies of responses of an untrained normal control at baseline with repeated responses taken 20 minutes after the first recording, analysed by repeated

measures analysis of variance (ANOVA). Figure 4.5 shows the two hexagonal trace arrays and the overall responses (superimposed large waveforms) of the baseline results (bold lines) and the repeated results (dashed lines). Some drift of waveform responses was evident because of the inexperience of the subject and the low cut-off filter value chosen (1 Hz). Choosing a higher cut-off (3 Hz) (Keating et al. 1996) which may eliminate drift would also decrease signal amplitude (Keating et al. 1996). For the short term repeatability results shown in Figure 4.5 local latencies did not differ significantly ($F_{(2,40)}=0.4$, $p=0.52$) while the mean amplitudes showed a 13% decrease ($F_{(2,40)}=6.2$, $p=0.02$).

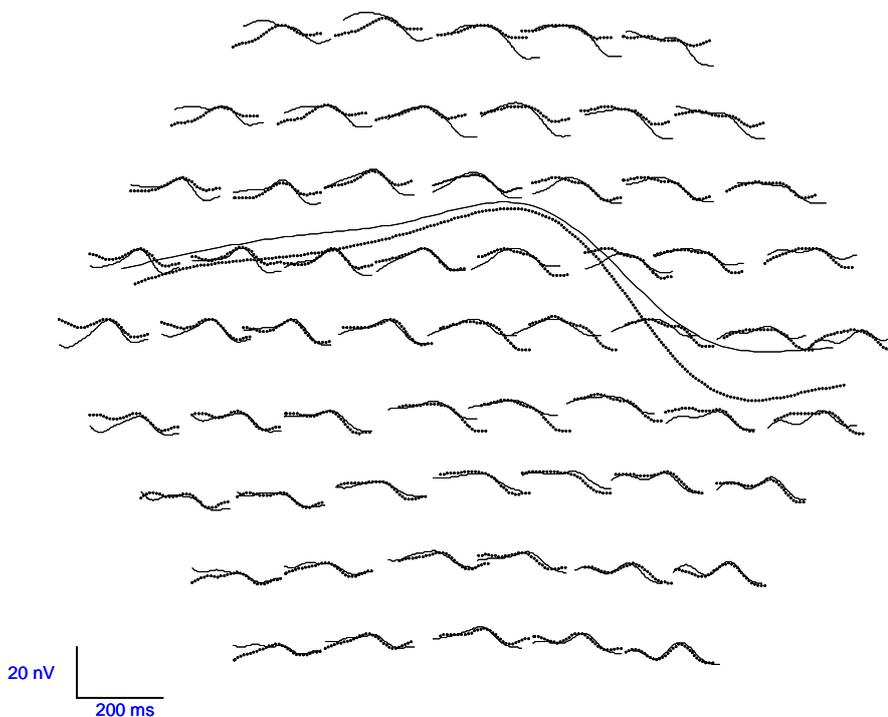


Figure 4.5. The hexagonal trace array (small waveforms) and the overall response (superimposed large waveform) of the rod-mediated mfERG of a control subject at baseline (solid lines) and repeated 20 minutes later (dashed lines).

We also compared the mean local amplitude and latency results of ten of our (untrained) control subjects at baseline and one year later (Figure 4.6). Again the local latency results showed no significant differences ($F_{(2,47)}=0.03$, $p=0.87$) between the two measurements suggesting it is a robust and relatively stable measure. We

found an increased mean amplitude by about 28% ($F_{(2,47)}=24.6$, $p<0.01$) which may be due to stray light influence which is thought to be greater in an older population according to Hood et al. (1998b). The scatter from increased lens opacities one year later together with the dilated pupil (larger than 8 mm) probably caused such an amplitude increase.

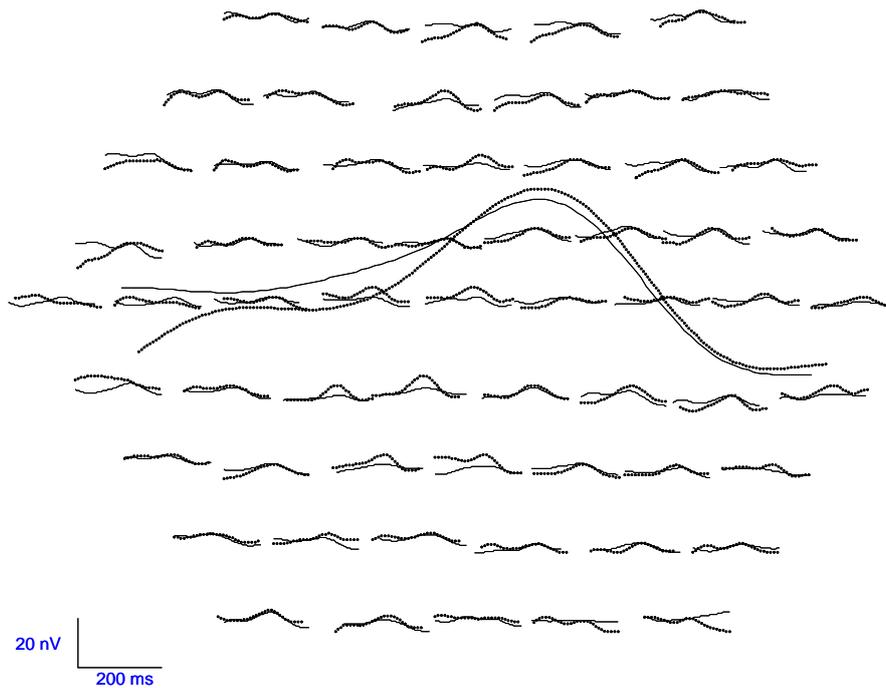


Figure 4.6. The trace array of the averaged rod-mediated mfERG responses from each hexagon (small waveforms) and the overall response (superimposed larger waveform) of ten control subjects at baseline (solid lines) and after one year (dashed lines). Although there are slightly greater amplitudes in the repeated measures the peak latencies show stable measures.

4.5 RESULTS

Significantly delayed central and inferior mean latencies were found for the rods in

Subjects (number of eyes)	Central mean (SD)	Peripheral (CP) mean (SD)	Superior mean (SD)	Inferior (SI) mean (SD)
Cones control (16)				
a-scale	1.05 (0.35)	0.99 (0.37)	0.98 (0.46)	1.02 (0.36)
t-scale	1.00 (0.07)	1.01(0.08)	0.99 (0.08)	0.99 (0.07)
Rods control (13)				
a-scale	1.61 (0.37)	1.8 (0.27)	1.78 (0.38)	1.77 (0.24)
t-scale	1.18 (0.19)	1.12 (0.13)	1.07 (0.09)	1.16 (0.16)
Cones ARM1 (11)				
a-scale	0.84 (0.29)	0.89 (0.22)	0.85 (0.31)	0.91 (0.25)
t-scale	0.94 (0.14)	0.98 (0.08)	0.95 (0.12)	1.0 (0.09)
Rods ARM1 (9)				
a-scale	1.6 (0.36)	1.73 (0.33)	1.77 (0.37)	1.59 (0.22)
t-scale	1.43 (0.31) *	1.20 (0.20)	1.19 (0.16)	1.44 (0.38) *
Cones ARM2 (6)				
a-scale	0.88 (0.17)	0.82 (0.35)	0.83 (0.31)	0.88 (0.25)
t-scale	0.96 (0.08)	1.03 (0.12)	0.99 (0.11)	1.01 (0.10)
Rods ARM2 (5)				
a-scale	1.74 (0.21)	1.67 (0.43)	1.56 (0.24)	1.56 (0.24)
t-scale	1.36 (0.16)	1.19 (0.11)	1.13 (0.10)	1.33 (0.32)

the ARM1 group compared to the control group (Tables 4.3 and 4.4).

Table 4.3. The means and standard deviations (SD) for the a- and t-scales for the central-peripheral (CP) and superior-inferior (SI) methods for the cones and rods of the control and ARM subjects
*p <0.01 statistically significant in comparison to the control group

Table 4.4. Rod results of the repeated measures analyses of variance for the CP and SI methods.

	CP method		SI method	
a-scale				
group effect	$F_{2,24}=0.01$	$p=0.99$	$F_{2,24}=0.09$	$p=0.55$
group x location	$F_{2,24}=0.51$	$p=0.61$	$F_{2,24}=1.23$	$p=0.31$
location effect	$F_{2,24}=0.42$	$p=0.52$	$F_{2,24}=0.03$	$p=0.86$
t-scale				
group effect	$F_{2,24}=4.88$	$p=0.02^*$ (between ARM1 and N-group, posthoc $p < 0.01^*$), (between ARM2 and N-group, posthoc $p = 0.07$)	$F_{2,24}=4.91$	$p=0.02^*$ (between ARM1 and N-group, posthoc $p < 0.01^*$)
group x location	$F_{2,24}=0.87$	$p=0.43$	$F_{2,24}=0.79$	$p=0.47$
location effect	$F_{2,24}=6.57$	$p=0.02^*$ (all groups)	$F_{2,24}=7.82$	$p=0.01^*$ (all groups)

*statistically significant

There was a similar trend for the ARM2 group but this did not reach statistical significance ($p = 0.07$). Although the cones showed a trend of lower mean a-scales for both ARM groups for both methods this was not statistically significant (Table 4.3).

Figure 4.7 shows the trace arrays for the cones (left) and rods (right) of one subject (subject h). The cone responses are within the normal range but show some misshapen waveforms while the rods show impaired results centrally, paracentrally and in the inferior field.

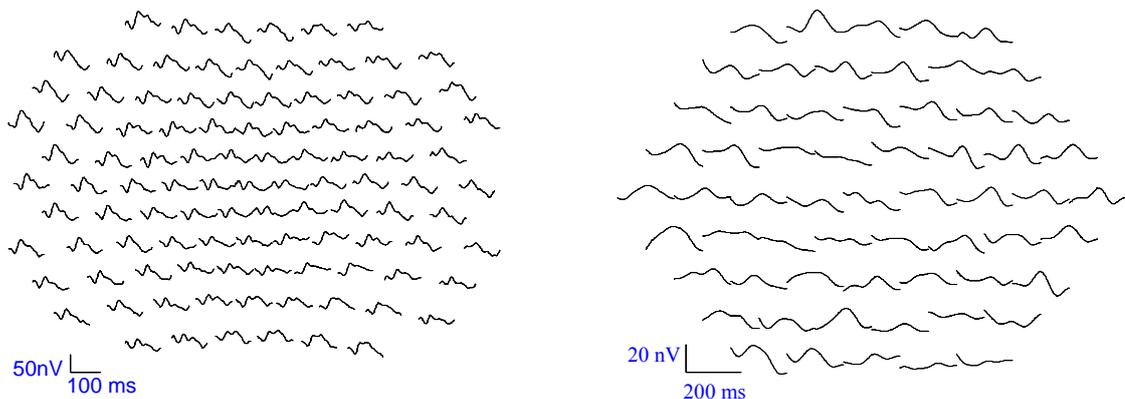


Figure 4.7. The cone- (left) and rod-mediated (right) mfERG trace arrays from one ARM subject (subject h) right eye. Impaired results are seen for the rods centrally, paracentrally and inferiorly while the cone results are misshapen but within the normal range. Note the different scaling for the cone- and rod-mediated traces. Drift is evident because of the 1 Hz lower bandpass cut-off used in the recording.

Repeated measures analyses of variance (ANOVA) of the mean a- and t-scales for the cones (Table 4.5) showed no statistically significant difference between ARM groups compared to the control group in either CP or SI methods (group effect). However the mean t-scales for the rods (Table 4.3, Figure 4.8) of the ARM1 group were significantly delayed compared to the control group in both methods (CP: $F_{2, 24}=4.88$, $p=0.02$, ARM1: posthoc, $p<0.01$, SI: $F_{2, 24}=4.91$, $p=0.02$, posthoc, $p<0.01$).

Table 4.5. Cone results of the repeated measures analyses of variance for the CP and SI methods.

	CP method		SI method	
a-scale				
group effect	$F_{2,30}=1.15$	$p=0.33$	$F_{2,30}=0.14$	$p=0.55$
group x location	$F_{2,30}=1.86$	$p=0.17$	$F_{2,30}=0.07$	$p=0.93$
location effect	$F_{2,30}=0.88$	$p=0.36$	$F_{2,30}=1.68$	$p=0.21$
t-scale				
Group effect	$F_{2,30}=0.88$	$P=0.42$	$F_{2,30}=0.19$	$P=0.82$
group x location	$F_{2,30}=1.39$	$p=0.26$	$F_{2,30}=0.84$	$p=0.44$
location effect	$F_{2,30}=6.47$	$p=0.02^*$ (all groups)	$F_{2,30}=2.79$	$p=0.11$

*statistically significant

Neither the ARM1 nor ARM2 groups' cone or rod responses had amplitude or latency estimates by location which were different from those of the controls but there was a significant location effect for the mean of the t-scales for the three groups (Tables 4.4 and 4.5). Shorter latencies were found centrally than peripherally for the cones ($F_{2,30}=6.47$, $p=0.02$) and there were longer central (CP: $F_{2,24}=6.57$, $p=0.02$) and inferior latencies (SI: $F_{2,24}=7.82$, $p=0.01$) compared to peripheral and superior latencies for the rods for the three groups (Fig. 4.8).

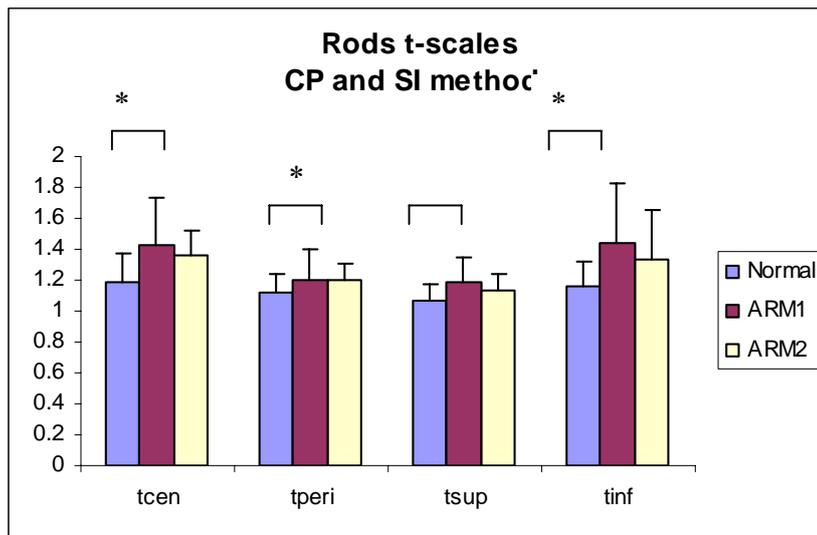
* $p < 0.01$

Figure 4.8. The mean and SD for the t values (note that this value is a ratio) for the rods showed significantly delayed latencies for the ARM1 group compared to the control group. There was also a trend to longer latencies for the ARM2 group which was not significant. For all groups latencies were longer centrally and inferiorly compared to the peripheral and superior field.

4.6 DISCUSSION

We investigated cone and rod functions in early ARM with the mfERG by using a computerized curve fitting method. Our data indicate a functional impairment of the rods which is consistent with Curcio's histopathological findings in early ARM (Curcio 2001). We found significantly delayed rod latencies in the ARM1 group in both methods. Although there was a trend toward longer mean rod latencies in the ARM2 group compared to the control group, this was not statistically significant. Thus, we could not discriminate between the different levels of early ARM with the rod-mediated mfERG.

In all groups the mean rod latencies were significantly delayed centrally (central averaged hexagons up to 3 mm) compared to peripherally (Table 4.4) which is in accordance with histopathological findings in aging and early ARM (Curcio et al. 1993; Curcio 2001). Curcio et al. (2001) detected a paracentral rod loss (0.5-2mm from the fovea) in aging and a rod loss particularly 0.5-1mm from the foveal centre in early ARM. In contrast to the findings of Curcio et al. (1993), we did not find a predilection for the lower retina reflecting the beginning rod impairment, but we found more compromised retinal function in the upper retina (inferior field) for all groups (Table 4.4). This might be due to the older ARM subjects (on average 70 years) we chose, as Curcio et al. (1993) described their early findings in a younger age group (between 44 and 58 years). Further this could reflect a beginning rod impairment in our subjects as Curcio et al. (1993) found the highest rod density 4-5 mm above the fovea in their healthy eyes.

For the cones, all three groups showed significantly shorter mean latencies centrally than peripherally (Table 4.5). This was more evident, but not statistically significant for the ARM groups. Seeliger et al. (1998b) investigated the implicit time topography of mfERG in subjects with normal vision and fundi and in subjects with retinitis pigmentosa. They found longer latencies centrally compared with the more peripheral retinal areas in their normal group by using a 61 hexagon stimulus and the concentric averaging method. They suggested that retinal asymmetry is evident due to physiological differences which are presumably at the level of cones. Seeliger et al. (1998b) further suggested that it was important to be aware of normal implicit time topography especially at the onset of maculopathy. If our finding of shorter mean central latencies reflects a beginning functional impairment for the cones then monitoring latencies across the retina might help in estimating the progression of early ARM.

Previous authors (Li et al. 2001) found reduced cone-mediated amplitudes and delayed latencies in early ARM by choosing concentric ring averaging methods. Li et al. (2001) described their ARM subjects as having early macular drusen or irregular pigmentation with RPE changes and a visual acuity of 6/8.5 or better. Although we found significantly faster latencies for all rings for the cones in the ARM1 group by applying their method of averaging the responses to six concentric rings, we found no amplitude reduction compared to the control group using the same method (data not shown). These findings are probably due to different ARM levels and age in their subjects compared to ours. However, in agreement with Li et al. (2001), we found no statistically significant differences between the superior and inferior fields mean amplitude and latency results.

Hood et al. (1998b) had noted in their studies with the rod-mediated mfERG that the stimulus and surround conditions that best isolated rod records still needed to be determined and may depend on the particular set up or even the patient population. They suggested that control experiments needed to be performed especially in older populations due to a possible greater stray light influence. We included the suggested stimulus conditions of Hood et al. (1998b) and adjusted them to our laboratory conditions and our older subjects including several surround and luminance control experiments. We slowed the stimulation sequence by inserting three blank frames which has been suggested for clinical use (Hood et al. 1998b). Although the amplitude becomes greater as the number of blank frames is increased the signal-to-noise ratio with three frames has been shown to be as good as with 14 frames inserted (Hood et al. 1998b). We overcame the stray light problems by adjusting surround luminance and by lowering the luminance of the stimuli. As previously reported (Hood et al. 1998b) we found larger peripheral responses due to stray light when no surround was added (Fig. 4.2). Unlike Hood et al. (1998b) who used Burian-Allen electrodes we used DTL electrodes which gave poorer signal-to-noise ratio (Esakowitz et al. 1993) but more comfort to our subjects. One of the major problems for our subjects was to overcome eye drift and maintain concentration and fixation especially in the rod protocol in which vision was indistinct. Besides breaking up the recording (32 segments), we also used a fixation cross extending to each corner of the screen which considerably aided fixation. Despite applying Hood et al. (1998b) suggested clinical protocol and the attempt to make it easier for the subjects (DTL electrodes, larger fixation cross, and shorter examination time) many of our subjects still had difficulty controlling eye movements, resulting in more waveform drifts. Thus, the rod-mediated response quality was worse than that of the

cone records, a fact that was also found by Hood et al. (1998b). It therefore still remains a demanding procedure that might not be suitable for every subject.

However, we were able to show that the local rod latency did not change significantly during either short or long term follow up in our control experiments. This demonstrates the reliability of our findings in ARM subjects in which only the latencies were significantly prolonged. The local rod-mediated mfERG amplitudes were less stable and showed more variation. Similar findings have been demonstrated for the local amplitudes of the cone-mediated mfERG by Fortune et al.(1999). They showed that local cone-mediated mfERG responses differed in amplitude by a factor of about 10 between eyes and about 5 within eyes in normal subjects whereas the variability of the latency was small suggesting it to be highly reliable.

The reason that the rods are first affected in ARM is unclear. Recent human and animal studies in ARM eyes have found a loss of photoreceptors, RPE cells and inner nuclear layer cells by apoptosis, preferentially affecting the rods (Hinton et al. 1998; Del Priore et al. 2002; Dunaief et al. 2002; Gordon et al. 2002). Curcio and colleagues (2000; 2001) hypothesized a vitamin A deficiency and therefore a retinoid deficiency, resulting from a reduced translocation of the retinoids from the blood across Bruch's membrane due to the accumulated cell debris between Bruch's membrane and the RPE. Another reason for preferential rod rather than cone vulnerability might be their larger oxygen requirements (Arden et al. 1998), a reduced choroidal blood flow has been demonstrated in ARM (Pauleikhoff et al. 1990a; Friedman et al. 1995; Grunwald et al. 1998). Grunwald et al. (1998) found

abnormal choroidal circulation in early stages of ARM and suggested that ischemia plays a major role in the development of AMD. Friedman et al. (1995) demonstrated increased vascular resistance of the short posterior ciliary arteries in AMD and proposed this to be the result of decreased compliance and calibre of choroidal vessels. An association between reduced rod responses and hypoxia has been described in diabetes (Arden et al. 1998). It is known that ischemia plays an important role in the pathogenesis and progression of proliferative diabetic retinopathy (Ulbig and Kampik 1993). Dark adaptation studies in subjects with early diabetes have also shown effects on the rods (Greenstein et al. 1993). Additionally mfERG studies in subjects with and without diabetic retinopathy demonstrated reduced oscillatory potentials (Kurtenbach et al. 2000; Onozu and Yamamoto 2003) which are thought to be largely rod-mediated (Wu and Sutter 1995). Kurtenbach et al. (2000) showed delayed mfERG oscillatory potentials rather than amplitude reduction in their subjects without diabetic retinopathy. Although we used a different protocol in deriving our rod-mediated mfERGs we hypothesize that our findings of delayed responses might reflect hypoxia due to reduced blood flow. This might also cause a decrease in the supply of important metabolites (such as vitamin A) to the rod outer segments as Curcio et al. (2000) suggested in their hypothesis.

We were able to record the rod-mediated mfERG in early ARM subjects and to show a functional loss of rod response with the mfERG in early ARM which has not been described before. This might represent an earlier functional impairment for the rods than for the cones, but this needs to be confirmed by a longitudinal study of a larger sample of older eyes. Our results suggest that a follow up of rod as well as cone

function in older eyes may be predictive of early ARM and may assist in assessing progression or monitoring the effect of treatment for ARM.

4.7 Acknowledgements

The authors thank Dion Scott for helping to develop the statfit analysis using Matlab (Mathworks, Natick, MA).

4.8 REFERENCES (see Master Reference List)

Comment

The results of the follow up study reported in Chapter 5 were analysed with a modified version of the template stretching method. Thus the baseline templates for rods and cones used in the two studies of Chapter 4 and Chapter 5 are different, resulting in different a- and t-scale values which cannot be compared.

CHAPTER 5**Monitoring retinal function****in early age-related maculopathy: visual
performance after one year****5.1 ABSTRACT**

5.1.1 Purpose. To monitor visual performance in early age-related maculopathy (ARM).

5.1.2 Methods. We measured monocular visual function - high-contrast visual acuity (HC-VA), central visual fields (mean sensitivity, MS), colour vision (desaturated Panel D-15), Pelli-Robson (P-R) and cone- and rod-mediated multifocal electroretinograms (mfERG) in 13 ARM subjects and 13 age-matched control subjects with normal fundi at baseline and after one year. All had visual acuity of 6/12 or better. The mfERG data were compared to templates derived from the control group at baseline. We analysed the mfERG results by averaging the central and peripheral fields and the superior and inferior fields (CP and SI methods) and by calculating the local responses.

5.1.3 Results. The mean rod-mediated responses were significantly delayed in the ARM group for the CP ($p=0.04$) and the SI methods ($p=0.03$) at baseline compared to the control group. This did not change significantly after one year, whereas the mean cone-mediated responses were within the normal range at both times. Although the local analysis revealed lower amplitudes for the cone- and rod-mediated responses at baseline this was not found after one year and only the local rod-mediated latencies were delayed at both times ($p<0.01$). HC-VA, desaturated Panel D-15 and P-R were significantly worse in the ARM group ($p\leq 0.01$) at baseline but did not show further significant deterioration. Progressive fundus changes were found in only two subjects (18%).

5.1.4 Conclusion. Although there was significant impairment of retinal function in early ARM at baseline no further deterioration was evident after one year.

5.2 KEYWORDS

Multifocal electroretinogram, early age-related maculopathy, cone-mediated function, rod-mediated function, mfERG, monitoring

5.3 INTRODUCTION

Many aspects of retinal function can be altered in early ARM (Brown et al. 1986b; Collins 1986; Kleiner et al. 1988; Sunness et al. 1988; Eisner et al. 1991; Curcio et al. 1996; Midena et al. 1997; Owsley et al. 2000; Jackson et al. 2002b; Feigl et al. 2004a; Feigl et al. 2004b) There have been several studies showing impairment of the photopic and scotopic pathways in early ARM subjects (Owsley et al. 2000; Li et al. 2001; Jackson et al. 2002b; Falsini et al. 2003; Gerth et al. 2003; Phipps et al.

2003; Scholl et al. 2004). Phipps et al. (2003) demonstrated an alteration of cone adaptational kinetics by measuring the post-bleaching recovery time to various contrast multiples. Falsini et al. (2003) showed altered temporal cone flicker sensitivity using the focal ERG and could discriminate between different degrees of ARM based upon fundus changes. Gerth et al. (2003) and Li et al. (2001) tested cone-mediated function with the multifocal electroretinogram (mfERG) in early ARM and found the latencies to be very sensitive for objective detection of functional impairment. Scholl et al. (2004) showed that retinal areas of increased fundus autofluorescence corresponding to drusen had a greater loss of scotopic rather than photopic sensitivity. Whether cone or rod-mediated function is first affected still needs to be investigated but there is histopathological (Curcio et al. 1996) and psychophysical evidence (Jackson et al. 1998; Owsley et al. 2000) of a preferential rod vulnerability in early ARM. Curcio and colleagues (1996; 2000) demonstrated a parafoveal rod loss but a normal foveal cone mosaic and Owsley et al. (2001) showed delayed rod-mediated dark adaptation in early ARM. Owsley et al. (2000) further showed that mean scotopic sensitivity loss exceeded the magnitude of photopic sensitivity loss in 87% of their ARM subjects. Hood et al. (1998b) developed guidelines for eliciting a rod-mediated mfERG and applied it successfully in subjects with hereditary diseases. We adapted a similar protocol to our laboratory conditions and subjects, to show a significant delay in the mean rod-mediated responses in early ARM compared to a control group, whereas the mean cone-mediated responses were within the normal range (Feigl et al. 2004a).

The aim of this study was to assess and monitor cone and rod-mediated function with subjective and objective vision tests in early ARM at baseline and after one year. Our

hypothesis was that, given the evidence of preferential rod vulnerability, the best indicator of the vision function tests we used for detecting impairment and/or progression of early ARM was the rod-mediated mfERG.

5.4 METHODS

5.4.1 Subjects

From 33 subjects who were initially selected from the Optometry Clinic at the Queensland University of Technology (QUT), Brisbane or were referred by local ophthalmologists and seen at baseline, 26 subjects were followed up after one year. Of these, 13 subjects made up the early ARM group (7 female, 6 male, mean age 72 years) and 13 subjects comprised the age-matched control group (8 females and 5 males, mean age 70 years). Of the other seven subjects seen at baseline two developed a chorioretinal neovascularisation (CNV) in the tested eye, four were not able to return for private reasons and one subject had died. All subjects had a distance visual acuity of 6/12 or better and were phakic at both visits. They underwent a full clinical examination, including slitlamp and fundus photography. All subjects who were enrolled in the study gave written informed consent and the tenets of the Declaration of Helsinki and the requirements of the University Human Research Ethics Committee at QUT were followed.

Slitlamp photographs were taken for lens (Age-Related Eye Disease Study Research Group 2001b) and fundus grading (Age-Related Eye Disease Study Research Group 2001a) which were performed according to the templates of the Age-Related Eye Disease Study (AREDS) as previously described (Feigl et al. 2004a). Retinal changes were graded independently by two experienced observers (BF, PS) using a set of the

Wisconsin age-related maculopathy standards with example photographs and a grid with grading circles (AREDS Reading Center, University of Wisconsin, Madison) (Age-Related Eye Disease Study Research Group 2001a; Bressler et al. 2003). One observer (PS) was masked to the subjects' functional results and agreement was achieved in all subjects with judgments of the other investigator (BF, not masked). We defined early ARM as the presence of either hard or soft distinct and indistinct drusen greater than $63\mu\text{m}$ in size with or without retinal pigment epithelial (RPE) abnormalities. Both types of drusen have been shown to damage cone and rod inner and outer segments (Johnson et al. 2003). Table 5.1 shows the characteristics of each ARM subject and the AREDS fundus grading (Age-Related Eye Disease Study Research Group 2001a) results at baseline and after one year.

Table 5.1. Subject characteristics with visual acuities (VA), desat. Panel D-15 error indices (C-index, the higher the index the poorer the colour vision) and type of deficiency in brackets and fundus grading results at baseline and after one year.

subjects	age at first visit	eye	VA baseline	VA one year	C-index baseline	C-index one year	fundus grading baseline	fundus grading one year
a	66	re	6/6	6/7.5	1.12 (normal)	1.15 (normal)	LIIa	LIIa
b	72	re	6/12	6/6 ⁻¹	3.14 (Tritan)	2.94 (Tritan)	LIIIb	LIIIb
c	73	le	6/6 ⁻¹	6/9.5 ⁻¹	1.57 (normal)	1.22 (normal)	LIIc (3)	LIIc (3) (more pigment)
d	72	re	6/12	6/9.5 ⁻¹	2.61 (Tritan)	2.76 (Tritan)	LIIc (3)	LIIc (3)
e	73	re	6/6 ⁻¹	6/9.5 ⁻²	1.77 (Tritan)	2.72 (Tritan)	LIIc (1)	LIIc (1)
f	69	le	6/7.5 ⁻¹	6/9 ⁺²	1.51 (Tritan)	1.34 (Tritan)	L IIIb	L IIIb
g	71	le	6/7.5 ⁻²	6/9 ⁺²	2.93 (Tritan)	3.15 (Tritan)	LIIc (3)	LIIc (3)
h	73	re	6/7.5	6/7.5 ⁺²	1.7 (Tritan)	2.07 (Tritan)	L IIb	L IIb
i	77	re	6/7.5	6/7.5 ⁻²	1.73 (Tritan)	2.78 (Tritan)	L IIIb	L IIIb
k	67	re	6/9.5 ⁺¹	6/12 ⁺¹	3.22 (Tritan)	3.61 (Tritan)	L IIIb	LIIc (3) (new pigment)
l	73	re	6/9.5 ⁺¹	6/9.5 ⁺¹	not possible	not possible	L IIIb	LIIIb
o	77	re	6/9.5	6/9.5 ⁻²	2.92 (non polar)	2.39 (Tritan)	LIIc (3)	LIIc (3)
p	72	re	6/6 ⁻¹	6/9.5 ⁻¹	2.25 (Tritan)	3.02 (Tritan)	LIIc (3)	LIIc (3)

5.4.2 mfERGs

The mfERGs (VERIS I, EDI Inc, San Mateo, CA) were recorded under the same conditions as recently described (Feigl et al. 2004a). We recorded monocularly by using DTL thread-electrodes and optical correction for the stimulus viewing distance (50 cm). Pupils were maximally dilated (tropicamide 0.5% and phenylephrine 2.5%) and the subjects were instructed to watch the centre of the monitor. The frame rate of the hexagonal display was 67 Hz, and the hexagons flickered according to a pseudorandom binary m-sequence ($2^{13}-1$ steps in length). The stimuli for the cones and rods were a 103 (35.5 x 28 deg) and a 61 hexagon array (33 x 28 deg), respectively. Retinal signals were bandpass filtered (1-300 Hz) and amplified (Grass P5 amplifier, x 100000). Blinks or small eye movements causing ERG artifacts during the recording segments were detected online and those segments were rejected and re-recorded. We used a cross extending to each corner of the screen to help subjects maintain fixation and controlled fixation by observing the subjects' recordings online on a monitor and by analysing the region of the blind spot after the recordings. Kernel overlap was excluded by performing an overlap test for each protocol.

5.4.2.1 Cone-mediated mfERG

The luminance was 100 cd/m² for the white hexagons and 2 cd/m² for the black hexagons (all our luminance measures were made with a Topcon BM-7 luminance-colorimeter). We divided the recordings into 16 segments (each about 11 seconds) and four files for averaging were obtained from every subject (total recording time was about 10 minutes per eye, not including resting time between the segments).

5.4.2.2 Rod-mediated mfERG

Rod-mediated mfERGs were recorded after 40 minutes dark adaptation using our protocol (Feigl et al. 2004a) (61 unscaled hexagons, Wratten 47B with ND filters and slowed stimulus sequence by inserting 3 blank frames) following the recommendations of Hood et al. (1998b). The luminance levels were 0.0098 cd/m^2 for the bright, 0.0005 cd/m^2 for the dark hexagons and 0.004 cd/m^2 for the surround. Figures 5.1A and 5.1B show hexagonal trace arrays for the cone- (A) and rod-mediated (B) results with the averaged local responses of the age-matched control group. The superimposed waveforms indicate the overall responses. The rod-mediated responses are smaller and broader than those of the cones with late, less distinct peaks.

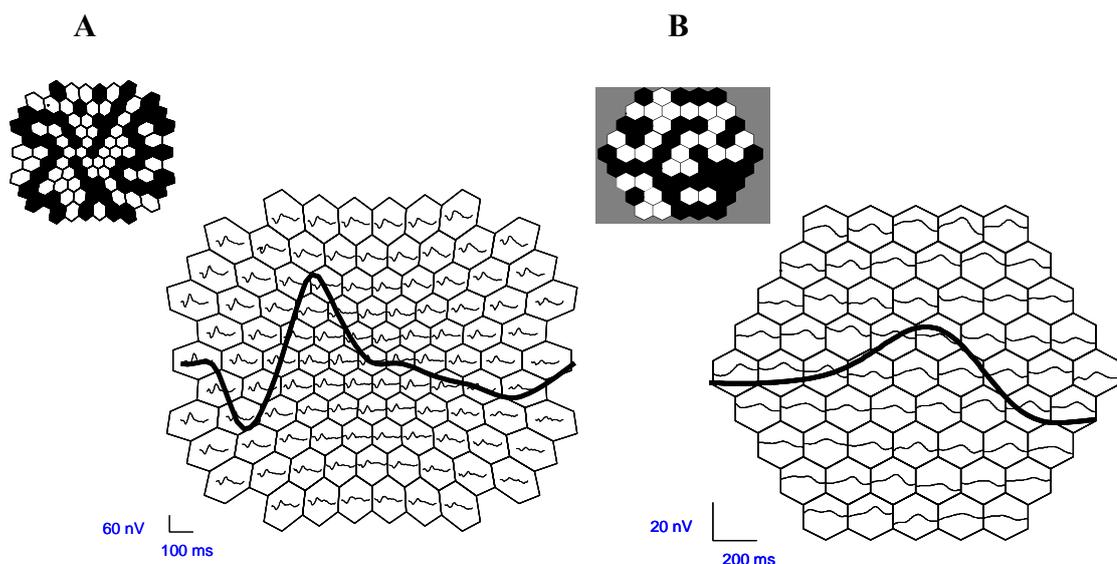


Figure 5.1A,B. The averaged cone (A)- and rod (B)-mediated mfERG responses of the age-matched control group with the hexagonal stimulus arrays above. The superimposed waveforms indicate the overall response. Note the different waveform shapes, with smaller and later rod-mediated responses without a distinct peak.

5.4.2.3 Analysis

We applied Hood and Li's (1997) computerized fitting method and derived normal templates for each of the 103 and 61 hexagons by averaging the local mfERGs recorded from the 13 control subjects at baseline. We then compared the control and

ARM subjects' baseline and one year amplitude (a-scale) and latency (t-scale) results against those templates. The quality of fit cut-off was set at 0.8 (Hood and Li 1997; Fortune et al. 1999; Holopigian et al. 2001; Holopigian et al. 2002; Feigl et al. 2004a; Han et al. 2004b) and results higher than 0.8 were considered as noise. The records of the left eyes were mirror-imaged so that appropriate parts of the retina were being compared across eyes.

Before fitting the cone- and rod-mediated responses, the data were spatially averaged once (ratio=6) and the VERIS noise reduction procedure (NRP) was performed (Sutter and Tran 1992). Signals were lowpass filtered at 80 Hz (cones) and 15 Hz (rods) resulting in better measurable signals for the fitting method without appreciable amplitude loss; cone and rod data were fitted over 55 ms (from 15 ms to 70 ms) and 120 ms (from 60 ms to 180 ms), respectively to avoid early and late transients.

We applied the previously described methods (Feigl et al. 2004a) and analysed the results by comparing the averaged central (cen) and peripheral (peri) (CP method) as well as superior (sup) and inferior (inf) (SI method) a and t-scales. We further analysed the a- and t-scales of each of the 103 and 61 local responses.

Cone-mediated mfERGs were analysed from 13 ARM eyes and from 13 control eyes at baseline and after one year. Rod-mediated mfERGs were analysed from 11 ARM eyes and 11 control eyes (ARM subjects l and p and two control subjects could not perform the rod mfERG procedure at both times) at baseline and after one year.

5.4.3 Psychophysical tests

Vision function tests were performed monocularly using standardized and recommended procedures at baseline and after one year. All subjects underwent the following tests: high contrast distance visual acuity (HC-VA, Bailey-Lovie charts), (Bailey and Lovie 1976) contrast sensitivity (Pelli-Robson), (Pelli et al. 1988; Elliott et al. 1990) colour vision (Lanthony desaturated D-15, desat. D-15) (Lanthony 1978; Committee on Vision 1981) and central visual fields (10-2 threshold, Humphrey-Zeiss 630, San Leandro, CA). Baseline results have been published in part previously (Feigl et al. 2004b).

5.4.4 Statistical analysis

Analyses comparing mean and local cone- and rod-mediated results were performed using analysis of variance (ANOVA) techniques as computed by the Statistical Package for the Social Sciences (SPSS-11). Repeated measures analyses of variance (ANOVA) within subjects (time, retinal location) and between subjects (group) were conducted.

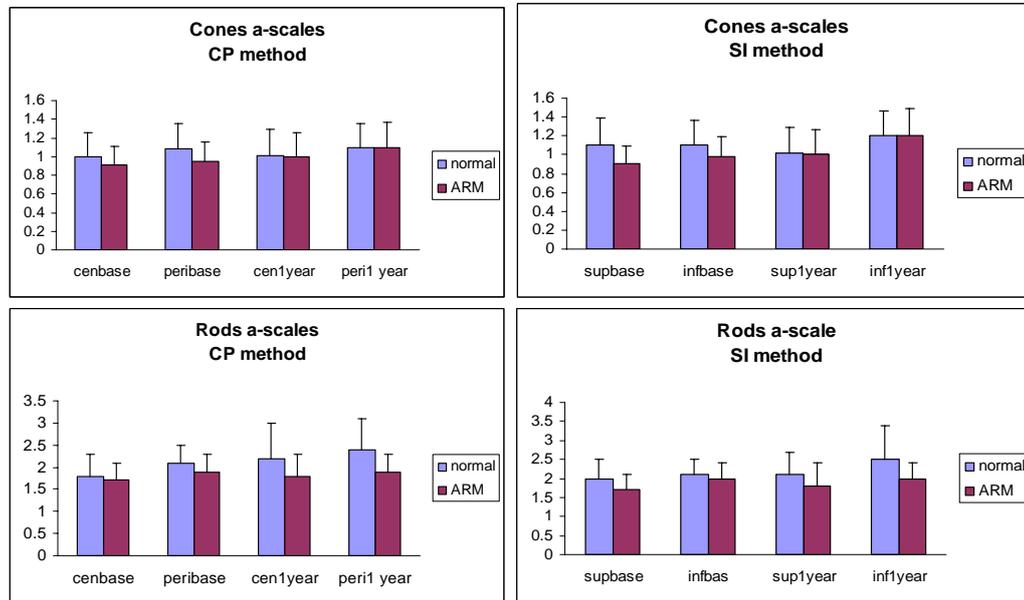
5.5 RESULTS

5.5.1 Mean cone- and rod-mediated mfERG results

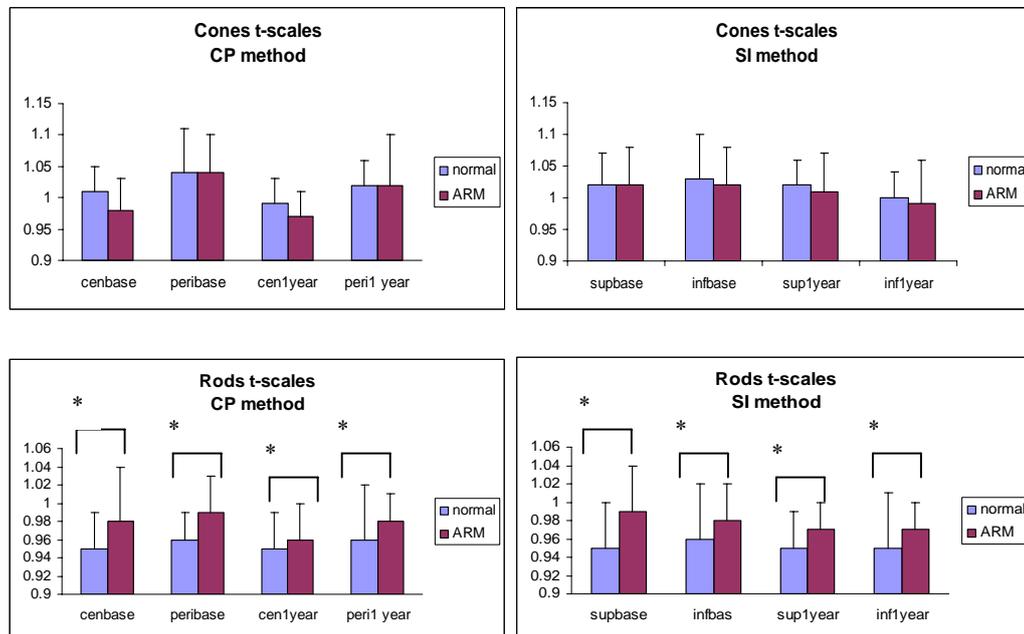
On average there was a trend of lower mean a-scales for the ARM group compared to the control group for both cone and rod-mediated responses for the CP and SI methods but this was not significant for either time (Figure 5.2A). For the mean t-scales significantly delayed latencies ($p \leq 0.04$) were evident for all locations for the rod- but not for the cone-mediated results at both times (Figure 5.2B). Figure 5.3

shows the trace arrays of the rod-mediated mfERG for the control group and the delayed waveforms for the ARM group at baseline and after one year with the averaged overall responses on the right hand side.

A



B



* $p \leq 0.05$

Figure 5.2A,B. **A.** The mean a-ratios (ordinate) for the central and peripheral (CP method) and for the superior and inferior field (SI method) at baseline (cenbase, peribase, supbase, infbase) and after one year (cen1year, peri1year, sup1year, inf1year) for the cone-(upper graphs) and rod-mediated (lower graphs) mfERG. Although there were slightly lower mean a-scales for the ARM group compared to the control group this was not statistically significant. **B.** The mean t-scale ratios for both methods show significantly delayed rod and slightly faster but not significantly cone-mediated latencies for the ARM group for both methods. Error bars indicate SD.

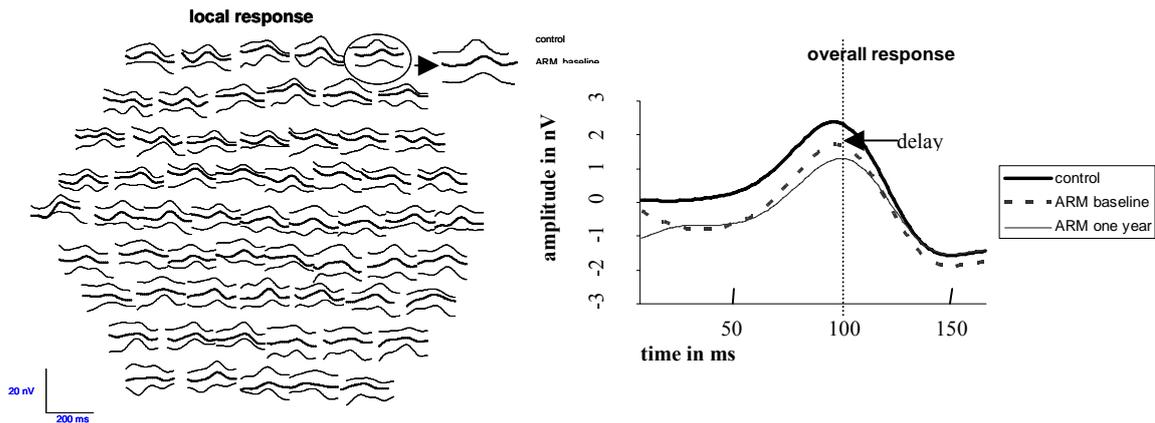


Figure 5.3. The local responses of the rod-mediated mfERG for the control (upper waveform), the ARM group at baseline (middle waveform) and after one year (lower waveform) showing delayed responses for the ARM group. On the right hand side the overall responses with delayed latencies for the ARM group at baseline and after one year compared to the control group are shown.

The repeated measures ANOVA for the mean a- and t-scales for the cones showed no significant effect for both methods. There was no significant group effect and no significant interaction between the groups by time or by locations. The rod-mediated mfERG demonstrated a significant group effect with longer latencies for the ARM group compared to the control group for both methods at baseline and after one year (CP method: t-scale: $F_{(1,20)}=5.08$, $p=0.04$; SI method: $F_{(1,20)}=5.6$, $p=0.03$). However, no significant interactions were found.

5.5.2 Local cone- and rod-mediated mfERG results

Although there were significantly lower amplitudes for the local cone-mediated responses for the ARM group compared to the control group at baseline ($p<0.01$), amplitudes were within the normal range after one year and this was reflected in a significant group by time interaction (Table 5.2, Figure 5.4). A significant group effect demonstrated faster local latencies for the ARM group at baseline and after one year ($p=0.03$) compared to the control group but no significant group by time interaction was evident here.

Table 5.2. Results of the repeated measures analyses of variance for the local cone-mediated mfERG responses.

local (103) responses	
a-scales	
group effect	$F_{(1,102)}=36.4$ $p<0.01^*$ (between ARM and control group at baseline)
group x time	$F_{(1,102)}=63.8$ $p<0.01^*$ (between ARM at baseline and ARM after one year)
t-scales	
group effect	$F_{(1,102)}=5.2$ $p=0.03^*$ (significantly faster latencies between ARM and control group at baseline and after one year)
group x time	$F_{(1,102)}=1.1$ $p=0.3$
*statistically significant	

There were lower local amplitudes for the rod-mediated responses of the ARM group compared to the control group at both times, but these were only significant after one year ($p<0.01$). A group by time interaction revealed that this significant difference was due to higher amplitudes for the control group after one year (Table 5.3, Figure 5.4).

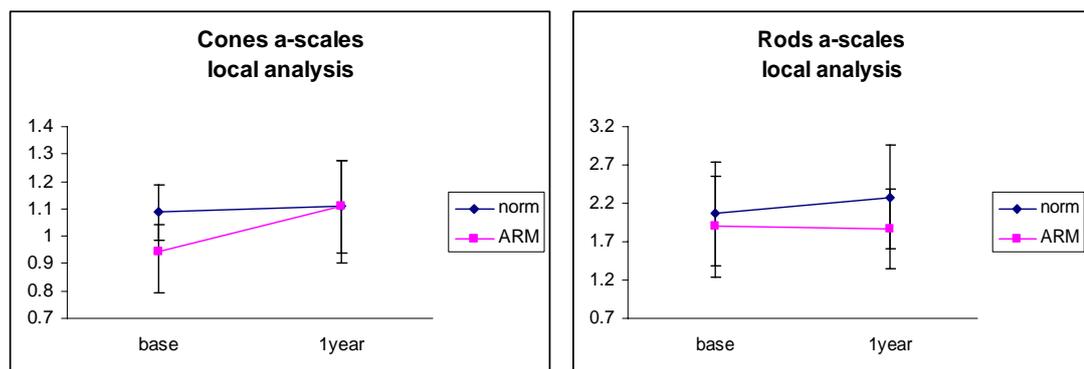


Figure 5.4. The means and standard deviations of the 103 and 61 hexagons for the a-scales of the local cone- and rod-mediated analysis are shown. A significant group by time interaction for the ARM and control group (norm) was evident. The ARM group demonstrated significantly higher cone-mediated a-scales after one year compared to baseline results and thus values comparable to the control group (left graph). Similarly the rod-mediated responses of the control group showed a significant increase for the a-scales after one year compared to baseline (right graph). Error bars indicated SD.

Further the local rod-mediated latencies showed a significant group effect with delayed latencies for the ARM group compared to the control group at both times ($p < 0.01$). No group by time interaction was evident (Table 5.3).

Table 5.3. Results of the repeated measures analyses of variance for the local rod mediated responses.

local (61) responses	
a-scales	
group effect	$F_{(1,60)}=19.8$ $p < 0.01^*$ (between ARM and control group after one year)
group x time	$F_{(1,60)}=6.1$ $p = 0.02^*$ (between control group at baseline and after one year)
t-scales	
group effect	$F_{(1,60)}=13.6$ $p < 0.01^*$ between ARM and control group at both times
group x time	$F_{(1,60)}=2.6$ $p = 0.1$
*statistically significant	

5.5.3 Psychophysical results

HC-VA, contrast sensitivity and desaturated colour vision (mainly Tritan defect, Table 5.1) were significantly impaired in the ARM group at baseline compared with the control group ($p \leq 0.01$) but did not change over time, while central visual fields were within the normal range at both times (Table 5.4). The results of the repeated measures ANOVA show a significant group effect for the HC-VA ($F_{(1,24)}=20.1$, $p < 0.01$), the Pelli Robson ($F_{(1,24)}= 16.5$, $p < 0.01$) and the desaturated Panel D-15 ($F_{(1,23)}=7.3$, $p \leq 0.01$) but no significant group by time effect. Thus these test results were significantly impaired in the ARM group compared to the control group at both times but showed no further deterioration after one year. MS was not significantly different between the groups at baseline or after one year.

Table 5.4. Mean results and standard deviations (SD) for the visual acuity (HC-VA), contrast sensitivity (Pelli), desaturated colour vision (desat. D-15) and central visual fields (MS) of the early ARM and control groups at baseline (ARM base, control base) and after one year (ARM one year, control one year)

	ARM base	ARM one year	control base	control one year
HC-VA (log Mar)	0.14 (± 0.11)*	0.18 (± 0.11)*	-0.01 (± 0.13)	0.0 (± 0.09)
Pelli (no. of letters)	32 (± 3)*	33 (± 3)*	36 (± 3)	35 (± 2)
desat D-15 (index)	2.04 (± 0.7)*	2.4 (± 0.8)*	1.5 (± 0.5)	1.8 (± 0.6)
MS (dB)	28 (± 2)	29 (± 2)	29 (± 3)	30 (± 2)

control one year)

* $p \leq 0.01$ significantly impaired compared to the control group at both times

5.5.4 Grading results

We found progressive fundus changes in only 2 (18%) subjects (c and k) who developed more or new retinal pigment epithelium (RPE) abnormalities (Table 5.1). Subject k was graded to a lower AREDS level (LII c3 from LIII b) because of new RPE abnormalities which have been shown to increase the risk of developing late ARM by about 3-fold (van Leeuwen et al. 2003b). None of the control subjects developed early ARM after one year.

5.6 DISCUSSION

Our results demonstrate impaired cone- and rod-mediated function at baseline with no further deterioration over a period of one year in the early ARM group. By using averaging methods (CP and SI) for the analysis of the mfERG results, a significant delay of the rod-mediated function was evident whereas cone function was within the normal range at both measurement times. Although the local analysis of the cone- and rod-mediated responses showed reduced amplitudes for the ARM group compared to the control group this was not the case after one year. However, consistent with the averaging method and our previous study (Feigl et al. 2004a) we

found delayed local rod-mediated and faster local cone-mediated latencies for the ARM group. Further, when we used a p value <0.01 , appropriate to the number of tests we performed, the local rod-mediated latencies together with the cone-mediated psychophysical measures appeared to detect deficits best. Thus our hypothesis that the rod-mediated mfERG would be the best of the tests we used for detecting functional impairment was not verified in our ARM sample.

The group by time effects showed higher local cone- and rod-mediated amplitudes after one year. This might be explained by the higher intra-individual variability for amplitudes (Seiple et al. 2003) than for latencies which has previously been shown for the cone-mediated mfERG. Additionally in our recent study we showed an amplitude increase for the normal control group by about 28% over a time period of one year but less variability in latencies for the rods (Feigl et al. 2004a). Another reason for higher amplitudes for the rods after one year may be a stray light influence which is thought to be greater in an older population according to Hood et al. (1998b). Variation in pupil size and retinal illumination can also have an influence on the mfERG responses (Chan and Brown 1998). Chan and Brown (1998) found that foveal mfERG amplitude increased with a slope of 5.24 nV/mm^2 of increased pupil area; this would predict an increase of about 120nV in mfERG amplitude as pupil size increased for example from 6 to 8 mm in diameter. However, as our subjects' pupils were maximally dilated at both visits and we do not expect larger pupils after one year especially in older subjects, we do not think this was the case in our findings. We therefore suggest greater reliance on latencies in detecting early changes than on amplitudes as they seem to be more robust. Delayed latencies in the cone-mediated mfERG have been shown to be early indicators in ARM (Gerth et al.

2003) and in other diseases such as diabetes and hereditary retinal conditions (Hood et al. 1998a; Fortune et al. 1999; Han et al. 2004b). Han et al. (2004b) demonstrated that new diabetic retinopathy was predicted by delayed latencies in an area without diabetic retinopathy a year before diabetic retinopathy developed. Whether local faster cone-mediated latencies for the ARM group compared to the control group reflect altered synaptic transmission and beginning pathology or not still needs to be investigated, but we did find delayed rod-mediated latencies in the local and averaging analysis methods at both times.

Curcio and colleagues (1993; 2001) demonstrated a selective vulnerability of parafoveal rods over cones in early ARM and a progressive rod loss of 30% throughout the lifespan. The reason that we could not show any significant change in rod-mediated function over time might be because the loss is very slow, affecting only about 2 rods/mm² per year (Curcio 2001). Curcio et al. (1993) further demonstrated that there were no gaps in the retina as the inner segments of the remaining rods expanded. It is possible that reduced rod function is compensated by the remaining rods at the beginning of the ARM disease process.

Fundus changes also did not progress in most of our subjects over this time period but slow progression in ARM has been described in other studies (Curcio et al. 1993; van Leeuwen et al. 2003b). The Rotterdam study (van Leeuwen et al. 2003b) has demonstrated a slow but constant progression in ARM severity with age and time following a distinct, stage by stage course. After two years the risk for developing the next stage of ARM was between 5% and 30%. Interestingly this was lowest (5%) for the most advanced stage but increased to about 40% after 6.5 years whereas less

advanced stages showed a progression rate to the next stage of less than 10% after 6.5 years. Given the slow progression rate, the short follow up of our subjects and the fact that most of our subjects had more progressed fundus changes to begin with (Table 5.1) our functional and morphological results seem reasonable.

Our study further is in accordance with findings of several other studies showing that colour vision (Collins 1986; Eisner et al. 1991; Midena et al. 1997) and contrast sensitivity (Brown and Lovie-Kitchin 1987a; Kleiner et al. 1988) are sensitive in detecting early impairment of cone function subjectively in ARM. Whether follow up with the rod-mediated mfERG over a longer period may indicate a significantly faster and greater rod-mediated impairment or not, as has been demonstrated with psychophysical tests (Jackson and Owsley 2000; Scholl et al. 2004), still needs to be investigated.

In future monitoring rod function might become an important issue in the treatment of ARM. Mohand-Said et al. (2001) found protective effects of transplanted rods on host cones in animal experiments. It has been hypothesised that this might help the survival of cones and that postponing or blocking death of rod cells, for example by the use of pharmacological agents, might provide future treatment options (Sunness et al. 1988; LaVail et al. 1992; Liu et al. 1999). However, given that the rod-mediated mfERG is a time consuming procedure (Hood et al. 1998b; Feigl et al. 2004a) shorter and less demanding protocols for assessing rod function would be more useful in the clinical setting. Further research is needed to establish if it is necessary to test close to the absolute threshold and if different colour or luminance backgrounds suppress or minimize cone function.

5.7 Acknowledgements

We thank Dion Scott for helping to develop the statfit analysis using Matlab (Mathworks, Natick, MA, USA) and Andrew Carkeet for helpful statistical assistance.

5.8 REFERENCES (see Master Reference List)

CHAPTER 6

Dynamics of outer retinal function after multiple photodynamic therapies in age-related macular degeneration: a pilot study

6.1 ABSTRACT

6.1.1 Purpose. To monitor outer retinal function between multiple laser treatments with photodynamic therapy (PDT) with the cone- and rod-mediated multifocal electroretinogram (mfERG) in eyes with age-related macular degeneration (AMD).

6.1.2 Design. Five observational case reports with electrophysiological and psychophysical studies.

6.1.3 Methods. Five eyes of five subjects with AMD were investigated before the first and one month after each of three PDT treatments. Function was assessed by using the cone- and rod-mediated multifocal electroretinogram (mfERG), high-contrast distance visual acuity (HC-VA), central visual fields (mean defect, MD, Humphrey 10-2) and contrast sensitivity (Pelli Robson, P-R). For each subject the local first-order mfERG results before treatment were used as a template and fitted against the local posttreatment results (Matlab, Mathworks).

6.1.4 Main outcome measures. Local cone- and rod-mediated first order mfERG amplitudes (a-scales) and latencies (t-scales).

6.1.5 Results. We found transient impairment of the cone- and rod-mediated amplitudes between the first and second treatment but there was stable or improved mfERG function in four of five eyes for the cone-mediated mfERG and in all eyes for the rod-mediated mfERG compared to baseline values after three treatments. One eye showed significant improvement and another showed reduction in the cone-mediated amplitudes. We found misshapen waveforms mainly affecting the descending part of the cone-mediated b-wave. Visual acuities and contrast sensitivities remained stable between the treatments in four and two eyes respectively, whereas central visual fields showed substantially higher MDs in two subjects after all treatments.

6.1.6 Conclusion. As found in previous studies of the cone-mediated mfERG after one PDT treatment, objective function was stabilized after multiple treatments in this small sample of AMD subjects. Similarly, rod-mediated function was not further compromised although there were poorer baseline responses with greater variability.

Precis. The local cone-and rod-mediated mfERG showed stable results after multiple PDT treatments. Transiently reduced amplitudes, possibly reflecting choroidal hypoperfusion were evident after one month. A larger sample size and control group are needed to confirm these findings and whether or not additional evaluation using electrophysiological criteria might be helpful in setting retreatment schemes.

6.2 KEYWORDS

photodynamic therapy, PDT, cone-mediated multifocal electroretinogram, rod-mediated multifocal electroretinogram, mfERG, age-related macular degeneration, AMD

6.3 INTRODUCTION

Photodynamic therapy (PDT) has been shown to stabilize visual acuity, contrast sensitivity, visual fields and fluorescein angiographic findings (Treatment of Age-Related Macular Degeneration With Photodynamic Therapy (TAP) Group 1999; Treatment of Age-Related Macular Degeneration With Photodynamic Therapy (TAP) Study Group 2001; Verteporfin In Photodynamic Therapy (VIP) Study Group 2001b; Palmowski et al. 2002; Rubin et al. 2002; Treatment of Age-Related Macular Degeneration With Photodynamic Therapy (TAP) Study Group 2002). Optimal treatment modalities such as time between dye injection and laser application, number of retreatments and interval between retreatments are still under investigation. Currently fluorescein angiography (FA) is the gold standard for following PDT laser treatment effects as well as in estimating retreatment schemes (Treatment of Age-Related Macular Degeneration With Photodynamic Therapy (TAP) and Verteporfin Therapy (VIP) Study Groups 2003). Guidelines for retreatment with PDT have been published but it has been acknowledged that current recommendations may be revised as more experience is gained (Treatment of Age-Related Macular Degeneration With Photodynamic Therapy (TAP) and Verteporfin Therapy (VIP) Study Groups 2003). Recent studies with indocyanine green angiography (ICG-A) indicate that the laser effect on the surrounding healthy choroid is underestimated (Michels and Schmidt-Erfurth 2003; Schmidt-Erfurth and

Michels 2003). Schmidt-Erfurth and Michels (2003) found substantial choroidal hypoperfusion after PDT treatments that did not resolve in time periods up to many months. They showed that increased size and density of the scotoma was associated with a more intense area of hypofluorescence due to collateral hypoperfusion which influenced the visual outcome. Schmidt-Erfurth et al. (2004) hypothesised that relative scotomas might be the consequence of PDT induced choriocapillary atrophy.

The first-order cone-mediated mfERG which mainly represents outer retinal function is capable of reflecting vascular deficits in central vein occlusion and diabetic retinopathy (Palmowski et al. 1997; Kretschmann et al. 2000; Hvarfner et al. 2003; Han et al. 2004b). Moreover it has been proven to be a valuable tool in documenting short and long term effects of one PDT on retinal function in several studies (Palmowski et al. 2002; Jiang et al. 2003; R  ther et al. 2003; Lai et al. 2004). Palmowski et al. (2002) showed an improvement of parafoveal function in nine of 16 eyes with the cone-mediated mfERG with varying retest intervals between two weeks and 15 weeks. R  ther et al. (2003) investigated 12 AMD eyes with predominantly classic choroidal neovascularisation before and an average of six weeks after PDT, and showed some further impairment which was not statistically significant. Jiang et al. (2003) monitored function 3 and 7 days post treatment and showed unchanged latency and amplitude densities in all six concentric rings of the mfERG measures. Most recently Lai et al. (2004) investigated the PDT effect using the mfERG on 14 eyes with idiopathic chorioretinal neovascularisations (CNV) and CNVs secondary to myopia, central serous chorioretinopathy and three eyes with CNVs due to AMD. They found transient reduction of the averaged central (0-7 degrees) and peripheral (7-25 degrees) amplitudes and delayed peak latencies four

days and two weeks after one PDT, but no difference from baseline results after one month (Lai et al. 2004).

However, none of these studies tested the rod-mediated mfERG and none investigated the cumulative effect of several PDT treatments on outer retinal function. Usually a number of laser treatments (on average about 4 in the first year) are required for up to three years after diagnosis of AMD. PDT damage to the choriocapillaris, the RPE and to the neurosensory retina might be possible. Histopathological studies in human eyes indicate no damage to the neural structures, cones, rods or the RPE after one PDT (Schloetzer-Schrehardt et al. 2002; Schmidt-Erfurth et al. 2002a) but no study has investigated treatment effects histopathologically after multiple PDT applications. In contrast, in animals some additional cell damage to the neurosensory retina can be detected after a single treatment (Zacks et al. 2002).

This study uses the cone- and rod-mediated mfERG to monitor local treatment effects on outer retinal function after multiple PDTs in contrast to previous studies which have analysed averaged cone-mediated mfERG responses after only one treatment (Palmowski et al. 2002; Jiang et al. 2003; Rütger et al. 2003; Lai et al. 2004). We were interested to determine how the surrounding paracentral and pericentral local areas were affected due to reported prolonged hypoperfusion and possible damage to the overlying neuroretina (Schmidt-Erfurth et al. 2002b; Wachtlin et al. 2003).

6.4 METHODS

6.4.1 Subjects

From seventeen potential PDT subjects referred by a local ophthalmologist five subjects (three female, two male) agreed to participate in the study. Their ages ranged between 64 and 81 years (mean 74 years). All subjects had subfoveal predominantly classic (> 50%) CNV confirmed by a fluorescein angiogram and met the criteria for primary PDT treatment (Treatment of Age-Related Macular Degeneration With Photodynamic Therapy (TAP) Group 1999). The pre-treatment visual acuities ranged from 6/24⁻² to 6/60⁺¹ and two subjects (3 and 4) were pseudophakic (Table 6.1).

Table 6.1. The subjects' characteristics and psychophysical measures

subject	age	eye	HC-VA	MD (dB)	P-R (letters)	follow up period after 1 st treatment (months)
1 pre			6/38 ⁻¹	-6.47	9	0
1 after 1 st PDT	64	RE	6/38 ⁺²	-4.78	16	1
1 after 2 nd PDT			6/48 ⁺¹	-5.58	14	4
1 after 3 rd PDT			6/38 ⁻¹	-6.31	11	6
2 pre			6/38 ⁺¹	-13.17	24	0
2 after 1 st PDT	74	RE	6/38	-11.65	20	1
2 after 2 nd PDT			6/60 ⁺¹	-12.76	15	2
2 after 3 rd PDT			6/48 ⁻²	-14.89	9	6
3 pre			6/60 ⁺¹	-14.27	x	0
3 after 1 st PDT	77	LE	6/48	-11.05	x	1
3 after 2 nd PDT			6/48	-13.24	x	2
3 after 3 rd PDT			6/60 ⁺²	-20.70	x	5
4 pre			6/48 ⁻¹	-8.0	6	0
4 after 1 st PDT	81	LE	6/48 ⁻²	-8.39	12	1
4 after 2 nd PDT			6/60 ⁺¹	-9.97	6	2
4 after 3 rd PDT			6/60 ⁺²	-9.68	2	4
5 pre			6/19	-3.95	24	0
5 after 1 st PDT	75	RE	6/24 ⁻²	-6.36	10	1
5 after 2 nd PDT			6/48 ⁻²	-7.8	6	3
5 after 3 rd PDT			6/48 ⁻²	-7.78	4	6

PDT treatments were performed according to the recommended procedures (Treatment of Age-Related Macular Degeneration With Photodynamic Therapy (TAP) Group 1999; Treatment of Age-Related Macular Degeneration With Photodynamic Therapy (TAP) Study Group 2001). Subjects were tested before the first treatment and one month after each treatment. This time period was chosen because most short term effects after treatments, such as increased intra- and subretinal fluid, are thought to have improved after one month (Treatment of Age-Related Macular Degeneration With Photodynamic Therapy (TAP) Study Group 2001; Rogers et al. 2002; Costa et al. 2003; Michels and Schmidt-Erfurth 2003). Subjects were not followed to the end of these PDT treatments and were still under retreatment consideration at the time this study was completed. All subjects showed membrane growth and activity based upon last FA findings and were scheduled to receive further treatments.

Informed consent was obtained from all subjects after explanation of the procedures. The study was conducted in accordance with the tenets of the Declaration of Helsinki and the requirements of the University Human Research Ethics Committee of the Queensland University of Technology.

6.4.2 Multifocal electroretinograms (mfERG)

The mfERGs (VERIS I, EDI Inc., San Mateo, CA) were recorded monocularly with DTL (Dawson-Trick-Litzkow) thread-electrodes; subjects were optically corrected for the stimulus viewing distance (50 cm). Pupils were maximally dilated with tropicamide 0.5% and phenylephrine 2.5%. The subjects were instructed to watch the centre of a monitor flickering between black and white hexagons.

For the cone-mediated mfERG the visual stimulus consisted of 103 scaled hexagons displayed on a monitor (width 35.5 deg, height 28 deg) driven at 67 Hz. The hexagons flickered according to a pseudorandom binary m-sequence ($2^{13}-1$) with a luminance of 100 cd/m^2 for the white hexagons and 2 cd/m^2 for the black hexagons (measured with a Topcon BM-7 luminance colorimeter). Recordings were divided into 16 segments (each about 10 seconds long). Kernel overlap was excluded by running an overlap test before setting up the protocol. Four recording files for averaging were obtained from every subject resulting in a total recording time of about ten minutes per eye (not including resting time between the segments). Retinal signals were band pass filtered (1-300 Hz), sampled every 1.87 ms and amplified (100,000 Grass amplifier). Blinks or small eye movements during the recording segments caused ERG artefacts which were detected online and such segments were rejected and re-recorded.

For the rod-mediated mfERG we applied a previously described method by Hood and colleagues (Hood et al. 1998b; Holopigian et al. 2001; Holopigian et al. 2002) which we have adjusted to our laboratory and applied in early ARM subjects (Feigl et al. 2004a; Feigl et al. 2004c). In brief we used a blue Wratten 47B filter (W47B) and neutral density filters (1.5 ND), and slowed the stimulus sequence by inserting 3 additional blank stimulus frames. The luminance conditions for the rod-mediated mfERG were 0.009 cd/m^2 for the bright, 0.0005 cd/m^2 for the dim hexagons and 0.004 cd/m^2 for the surround. We demonstrated repeatability of data in several control experiments (Feigl et al. 2004a). After 40 minutes of dark adaptation, the rod-mediated mfERGs were recorded with a stimulus array of 61 equally sized hexagons which flickered concurrently according to a pseudorandom binary m-

sequence ($2^{13}-1$ steps in length). One recording was obtained for each subject at each recording session. This was divided into 32 segments (each lasting about 21 sec) resulting in a total recording time of about 11 minutes (not including resting time between the segments).

Fixation was controlled by observing records online on the monitor and by analysing the blind spot. We used a large fixation cross extending to each corner so that maintaining fixation despite reduced central visual acuity was not a problem for most of our subjects. However, two of our subjects (4 and 5) preferred to use their better eye for fixation but recording was done monocularly.

Cone-mediated responses were derived before the first treatment and one month after each of three treatments in all subjects. Because of the demanding protocol (Hood et al. 1998b; Feigl et al. 2004a; Feigl et al. 2004c) rod-mediated mfERGs were recorded before and one month after each of only two treatments in all subjects (after 1st and 2nd PDT treatments).

6.4.3 mfERG analysis

For analysing the local first order kernel mfERG amplitudes and latencies we used a customized computer program (written in Matlab; Mathworks, Natick, MA) similar to that described by Hood and Li (1997). We have applied this method in early ARM subjects recently (Feigl et al. 2004a; Feigl et al. 2004c). It uses templates derived from the waveform responses of a representative group or subject which is manipulated in amplitude (a-scale) and time (t-scale) by a least squares method (statfit) to approximate the response signals from a given group or subject. A perfect

fit or statfit value of 0.0 is the result of an a-scale and a t-scale value of 1.0 which indicates that the values are equal to those of the template. For example an a-scale of 0.5 indicates a 50% lower amplitude or t-scale of 1.2 indicates a 20% longer latency than those of the template. This method has been shown to be sensitive in analysing and detecting small responses with low signal-noise ratio (Holopigian et al. 2001; Holopigian et al. 2002; Seiple et al. 2003; Feigl et al. 2004a; Feigl et al. 2004c; Han et al. 2004b). We used an individual's pre-treatment (baseline) values (a- and t-scales) for each of the hexagons as the templates and fitted those against each of the post-treatment a- and t- scales. A cut-off value for 'statfit' which is provided by this program, to discriminate signal from noise, was set at 0.8 based upon previously recommended values (Hood and Li 1997; Holopigian et al. 2001; Holopigian et al. 2002) and our own experience (Feigl et al. 2004a; Feigl et al. 2004c). Data which exceeded this value were regarded as noise and not included in the statistical analysis.

Before fitting the mfERG responses, the data were spatially averaged once (ratio=6) and the VERIS noise reduction procedure (NRP) was performed (Sutter and Tran 1992). For the fitting method signals were lowpass filtered at 80 and 15 Hz for the cones and rods, respectively without appreciable amplitude loss. Cone and rod data were fitted from 15 ms to 70 ms and from 60 ms to 180 ms respectively, to avoid early and late transients.

6.4.4 Statistical analysis of mfERG results

For each subject one repeated measures ANOVA was performed on the local responses for amplitudes and one repeated measures ANOVA on the local responses

for latencies across all visits. For the cones the local responses were each categorized by location: central, paracentral and pericentral. Each subject's local responses were analysed by number of treatments (4 including baseline) and by locations (3) and a two-way ANOVA examined interaction between these factors. Given the histopathological evidence of a great reduction of rods in late ARM (Curcio et al. 1996), only a one-way ANOVA was performed to examine the treatment effects on the local responses across all visits for the rod-mediated responses. Where there were significant findings paired t-tests were performed to examine the effects.

6.4.5 Psychophysical measures

A number of vision function tests were assessed monocularly with subjects optically corrected for the relevant test distances. All tests were performed less than a week before and one month after each treatment using the standardized and recommended procedures. Subjects were examined using the following tests: high-contrast (HC-VA) distance visual acuity measured with the Bailey-Lovie chart scored letter by letter (Bailey and Lovie 1980); contrast sensitivity measured using the Pelli-Robson letter contrast sensitivity chart (P-R) (Pelli et al. 1988) with letter by letter scoring (Elliott et al. 1990) and central visual fields (10-2 threshold) with the Humphrey Field Analyzer (Model 750i, Humphrey-Zeiss, San Leandro, CA) and mean defect (MD). Central 10 degree visual fields (resolution 4°) were performed to provide finer central resolution more in accordance with the mfERG resolution which can detect scotomas less than 4 degrees (Hood 2000). Other Humphrey programs (for example 30-2 or 24-2 threshold) which provide a better match to the mfERG area tested (35.5 degrees), have only 6° visual field resolution.

6.5 RESULTS

6.5.1 Cone-mediated mfERG

Functional stability or recovery was evident but misshapen waveforms were found in most of the subjects especially after the third treatment. The waveforms showed a broader shape with a double peak. Typically the descending part of the b-wave was affected suggesting dysfunction in ON and OFF bipolar recovery and hyperpolarisation (Hood et al. 2002; Greenstein et al. 2004) (Figs 6.1-3B).

Although the local responses were analysed, to simplify data presentation, results shown in Table 6.2 give the mean results (ratios indicating the relative change from the baseline values) with standard deviations (\pm SD) of the cone-mediated mfERG for the a- and t-scales of each subject after each treatment. A-scale values <1.0 indicate lower amplitudes whereas t-values >1.0 indicate longer latency compared to baseline (template) values for each subject.

Table 6.2. The mean results of the cone-mediated mfERG a- and t-scales of the central, para- and pericentral hexagons and the standard deviation (\pm SD) after one, two and three treatments (note the numbers are ratios compared to pre-treatment values of each subject).

	central a-scale	paracentral a-scale	pericentral a-scale	central t-scale	paracentral t- scale	pericentral t-scale
subject 1						
1 st PDT	0.5(\pm 0.4)*	0.7(\pm 0.3)*	0.9(\pm 0.3)*	1.1(\pm 0.3)	0.9(\pm 0.1)	0.96(\pm 0.1)
2 nd PDT	0.8(\pm 0.2)*	0.7(\pm 0.2)*	0.7(\pm 0.4)*	1.2(\pm 0.3)	0.98(\pm 0.2)	0.98(\pm 0.1)
3 rd PDT	0.9(\pm 0.5)	0.9(\pm 0.2)	0.9(\pm 0.5)	1.1(\pm 0.2)	0.8(\pm 0.2)	0.9(\pm 0.3)
subject 2						
1 st PDT	1.1(\pm 0.8)	1.1(\pm 0.4)	0.9(\pm 0.5)	0.9(\pm 0.2)*	0.9(\pm 0.1)	1.0(\pm 0.2)
2 nd PDT	0.9(\pm 0.7)	1.1(\pm 0.6)	1.0(\pm 0.4)	1.2(\pm 0.3)	1.0(\pm 0.2)	1.0(\pm 0.2)
3 rd PDT	0.9(\pm 0.5)	0.8(\pm 0.5)	0.9(\pm 0.4)	1.1(\pm 0.3)	0.95(\pm 0.1)	0.9(\pm 0.1)
subject 3						
1 st PDT	2.4(\pm 0.9)	2.8(\pm 1.1)+	3.0(\pm 1.1)+	0.9(\pm 0.1)	0.9(\pm 0.2)	1.0(\pm 0.1)
2 nd PDT	2.3(\pm 0.9)	2.8(\pm 1.0)+	2.9(\pm 1.0)+	0.9(\pm 0.1)	0.9(\pm 0.1)	0.9(\pm 0.1)
3 rd PDT	2.1(\pm 0.9)	3.2(\pm 1.1)+	3.2(\pm 0.8)+	0.9(\pm 0.1)	0.9(\pm 0.1)	0.9(\pm 0.1)
subject 4						
1 st PDT	0.7(\pm 0.3)*	0.9(\pm 0.3)*	0.7(\pm 0.3)*	1.0(\pm 0.1)	0.9(\pm 0.1)	1.0(\pm 0.1)
2 nd PDT	1.4(\pm 1.8)	1.1(\pm 0.6)	1.1(\pm 0.5)	1.0(\pm 0.2)	1.0(\pm 0.1)	1.0(\pm 0.2)
3 rd PDT	0.8(\pm 0.6)	0.9(\pm 0.5)	1.0(\pm 0.5)	0.9(\pm 0.1)	1.0(\pm 0.1)	0.9(\pm 0.3)
subject 5						
1 st PDT	0.5(\pm 0.2)*	0.5(\pm 0.1)*	0.6(\pm 0.1)*	1.0(\pm 0.1)	0.9(\pm 0.1)	1.0(\pm 0.1)
2 nd PDT	0.7(\pm 0.2)*	0.6(\pm 0.3)*	0.7(\pm 0.3)*	0.9(\pm 0.1)	1.0(\pm 0.1)	0.9(\pm 0.1)
3 rd PDT	0.3(\pm 0.3)*	0.5(\pm 0.3)*	0.7(\pm 0.4)*	1.4(\pm 0.3)+	1.1(\pm 0.3)+	1.1(\pm 0.3)+

* $p \leq 0.01$ decrease, + $p < 0.01$ increase from baseline values

A two-way ANOVA for each subject (Table 6.3) showed significant transient decreases (*) in the cone-mediated amplitude in two subjects (1, 4) between the PDT treatments. After three PDT treatments a significant decrease in amplitude was evident in only one subject (subject 5) in all locations while the others showed either stabilisation (subject 1, 2 and 4) or improvement (subject 3). A significant treatment by location interaction was evident for subject 3 showing improvement para- and pericentrally after all treatments (Fig 6.2B).

Table 6.3. Cone-mediated mfERG results of repeated measures ANOVA for each subject for the a-scales

Subjects	2-way ANOVA across all visits	outcome
subject 1		stable after 3 PDTs
treatment effect	$F_{(3,21)}=3.9, p=0.02^*$	after 1st and after 2nd PDT: $p<0.01^*$ after 3 rd : $p=0.1$
treatment x location	$F_{(6,42)}=1.1, p=0.4$	no significant interaction
subject 2		stable after 3 PDTs
treatment effect	$F_{(3,27)}=0.8, p=0.5$	no treatment effect
treatment x location	$F_{(6,54)}=0.6, p=0.7$	no significant interaction
subject 3		improved para and pericentrally after all 3 PDTs
treatment effect	$F_{(3,63)}=63.0, p<0.01+$	after 1st, 2nd and 3rd PDT $p<0.01$
treatment x location	$F_{(6,126)}=2.4, p=0.04+$	significant interaction para and pericentrally after 1st PDT: $p<0.01+$ after 2nd PDT: $p<0.01+$ after 3rd PDT: $p<0.01+$
subject 4		stable after 3 PDTs
treatment effect	$F_{(3,51)}=6.9, p<0.01^*$	after 1st PDT: $p<0.01^*$ after 2 nd PDT: $p=0.2$ after 3 rd PDT: $p=0.5$
treatment x location	$F_{(6,102)}=1.5, p=0.2$	no significant interaction
subject 5		impaired after all PDTs
treatment effect	$F_{(3,39)}=34.1, p<0.01^*$	after 1st, 2nd, 3rd PDT: $p<0.01^*$
treatment x location	$F_{(6,78)}=1.4, p=0.3$	no significant interaction

*significantly worse compared to baseline,

+significantly better compared to baseline

We found transiently significantly faster central latencies for subject 2 ($F_{(6,54)}= 3.1$, $p \leq 0.01$) after the first PDT treatment and delayed latencies for all locations for subject 5 ($F_{(3,39)}=7.6$, $p < 0.01$) after the 3rd PDT (Table 6.2).

6.5.2 Rod-mediated mfERG

As expected the rod-mediated mfERG showed great variability between the treatments and poorer signals compared to the cone-mediated results (Hood et al. 1998b; Holopigian et al. 2001; Holopigian et al. 2002) (Fig 6.1-3C). However, after the first and second PDT rod-mediated function showed results comparable to baseline for all subjects. Data were analysed locally across all visits but to simplify data presentation, mean overall results are shown in Table 6.4.

Table 6.4. The overall results of the rod-mediated mfERG a- and t-scales (indicating ratios relative to pre-treatment or baseline results of each subject) and the standard deviation (\pm SD) after treatments one and two.

	overall a-scale	overall t-scale
subject 1 1 st PDT 2 nd PDT	1.0(\pm 0.6) 1.0(\pm 0.5)	1.0(\pm 0.2) 0.9(\pm 0.2)
subject 2 1 st PDT 2 nd PDT	0.7(\pm 0.4) 0.8(\pm 0.4)	0.9(\pm 0.2) 0.9(\pm 0.3)
subject 3 1 st PDT 2 nd PDT	0.6(\pm 0.3)* 0.9(\pm 0.4)	0.9(\pm 0.2) 1.0(\pm 0.2)
subject 4 1 st PDT 2 nd PDT	1.0(\pm 1.0) 1.1(\pm 0.7)	0.9(\pm 0.2) 1.0(\pm 0.2)
subject 5 1 st PDT 2 nd PDT	0.7(\pm 0.5) 0.9(\pm 0.6)	0.9(\pm 0.2) 0.9(\pm 0.2)

* $p < 0.01$ (significantly worse compared to baseline)

Transiently decreased and/or misshapen waveforms were evident in three subjects (subjects 2, 3 and 4) while a significant transient decrease in amplitude was found only for subject 3 ($F_{(2,18)}= 3.7$, $p < 0.01$ after the 1st treatment (Table 6.4).

Figures 6.1-3A-C show the individual data of subjects 1, 3 and 5. For each example the early and late FA findings before the first therapy and after the 3rd PDT treatment are shown (A), the middle section with two rows demonstrates the cone-mediated mfERG trace arrays (B) and the rod-mediated responses are shown in the lower section (C). The filled and white hexagons of cone-mediated trace arrays indicate the local hexagons which were compared to the local baseline results. To illustrate the waveform shapes better the large superimposed waveforms indicate the averaged responses from the central (dark hexagons), para- (bright hexagons) and pericentral (white hexagons) local responses although results were analysed for each local response. The central 10° visual fields are shown on the right hand side of each of the central mfERG trace arrays and cover about the same area as the central responses of the mfERG (dark hexagons).

Figure 6.1A-C shows the results of the right eye from subject 1. (A) There was membrane growth (from 2.96 mm to 4.16 mm in diameter) with less density of the central scotoma (B) and no change in MD after three treatments (Table 6.1). A significant decrease in the cone-mediated amplitudes for all locations (B) was found after the 1st PDT and 2nd PDT but these recovered to non-significant results compared to baseline after the 3rd PDT (Tables 6.2 and 6.3). Waveforms were misshapen at baseline and between the treatments which was more evident after the 3rd PDT. The rod-mediated responses (C) showed no further significant deterioration through therapy compared to pre-treatment (Table 6.4).

In Figure 6.2A-C we demonstrate the significantly improved para- and pericentral cone-mediated mfERG results of subject 3 (Tables 6.2 and 6.3) despite increased

density of the central scotoma (Table 6.1) and membrane growth (3.76 mm in diameter at baseline to 5.83 mm in diameter after 3rd PDT). Interestingly rod-mediated responses (C) showed a significant decrease after one PDT but improved to baseline results and showed less misshapen waveforms after two treatments (Table 6.4).

Subject 5 results given in Figure 6.3A-C demonstrated steady deterioration of cone-mediated results as well as central visual fields (Table 6.1) and membrane growth (3.5 mm in diameter at baseline to 9.5 mm in diameter) after 3 treatments (A). This was significant for all local cone-mediated responses after the first, second and third treatments (Tables 6.2 and 6.3) compared to baseline results. Again more misshapen waveforms mainly affecting the descending part of the b-wave were evident after the 3rd treatment. Rod-mediated responses (C) showed reduced amplitudes especially after the 1st PDT, but the change in response was not significant compared to the baseline results (Table 6.4).

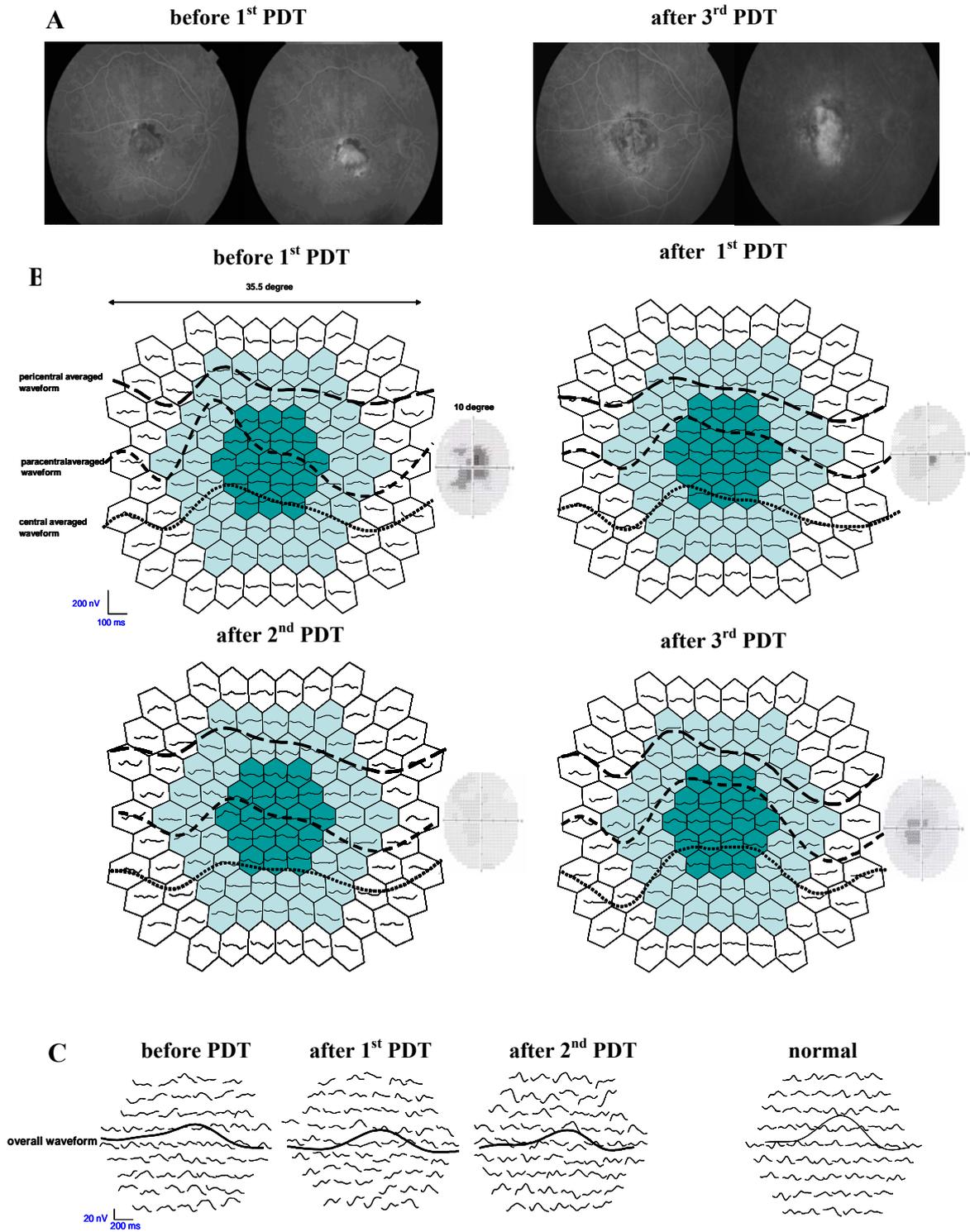


Figure 6.1A-C. (A) The early and late FA before and after 3 PDTs of the right eye of subject 1 shows membrane growth after three PDTs. (B) We found significantly reduced cone-mediated a-scales for all retinal locations after the 1st and 2nd PDT compared to the baseline results. Although misshapen waveforms were evident (especially after the 3rd PDT) these were comparable to baseline results and central visual field improved to baseline results after 3 PDTs. (C) Rod-mediated responses were still recordable at baseline and after two PDT treatments (superimposed large waveform indicates overall response). The rod-mediated mfERG responses of a healthy subject are shown for comparison on the very right side of this row.

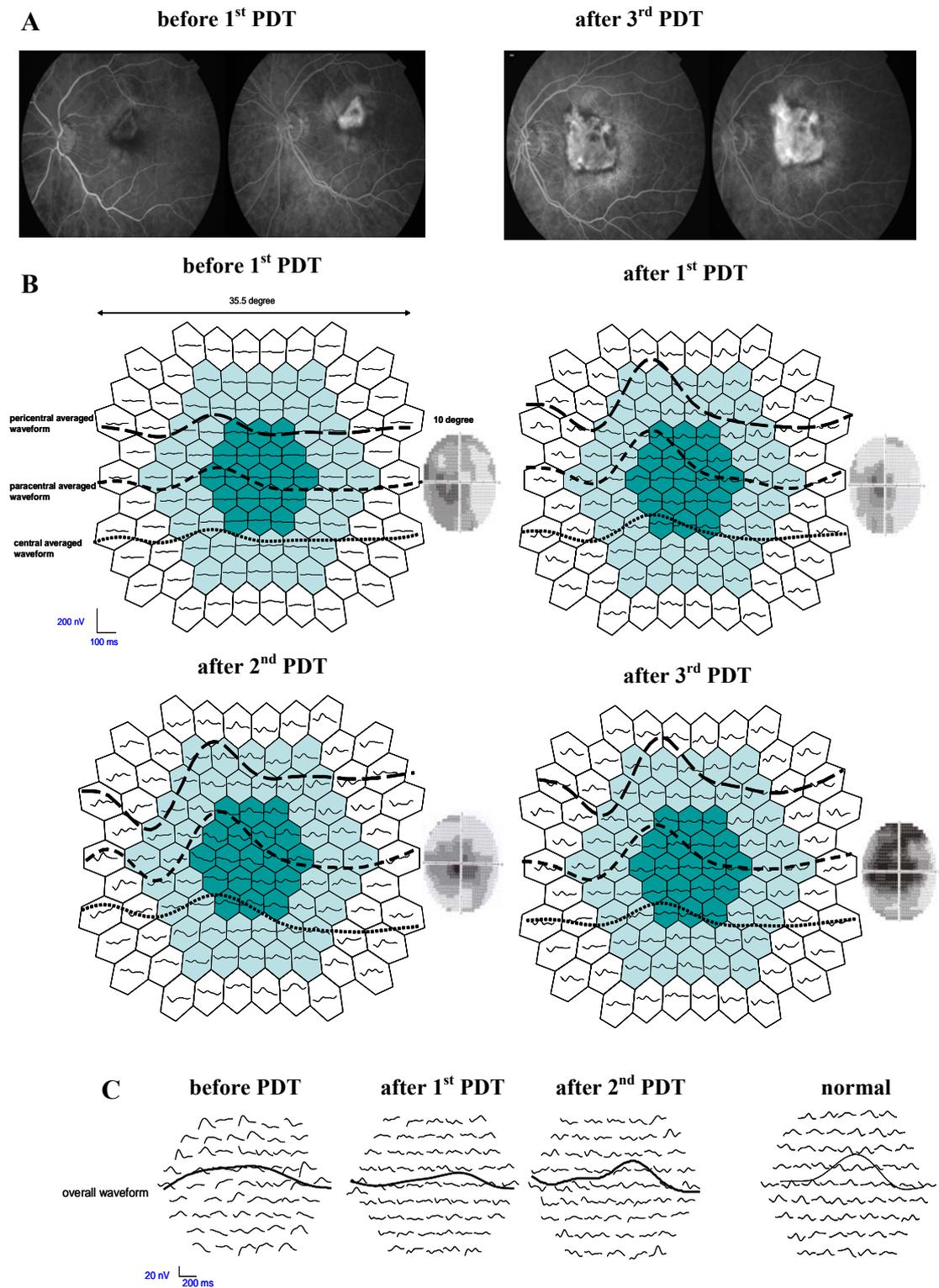


Figure 6.2A-C. (A) The early and late phase FA of the left eye of subject 3 before and after the 3rd PDT treatment. There was steady membrane growth and a deterioration of central (10°) visual field while local para- and pericentral cone-mediated mfERG responses (B) showed significantly higher a-scales after each treatment. (C) Significantly reduced rod-mediated a-scales were only evident after the first PDT but recovered to pretreatment values and were less misshapen after the 2nd PDT (superimposed large waveform represents the averaged overall response). The rod-mediated mfERG responses of a healthy subject are shown for comparison on the very right side.

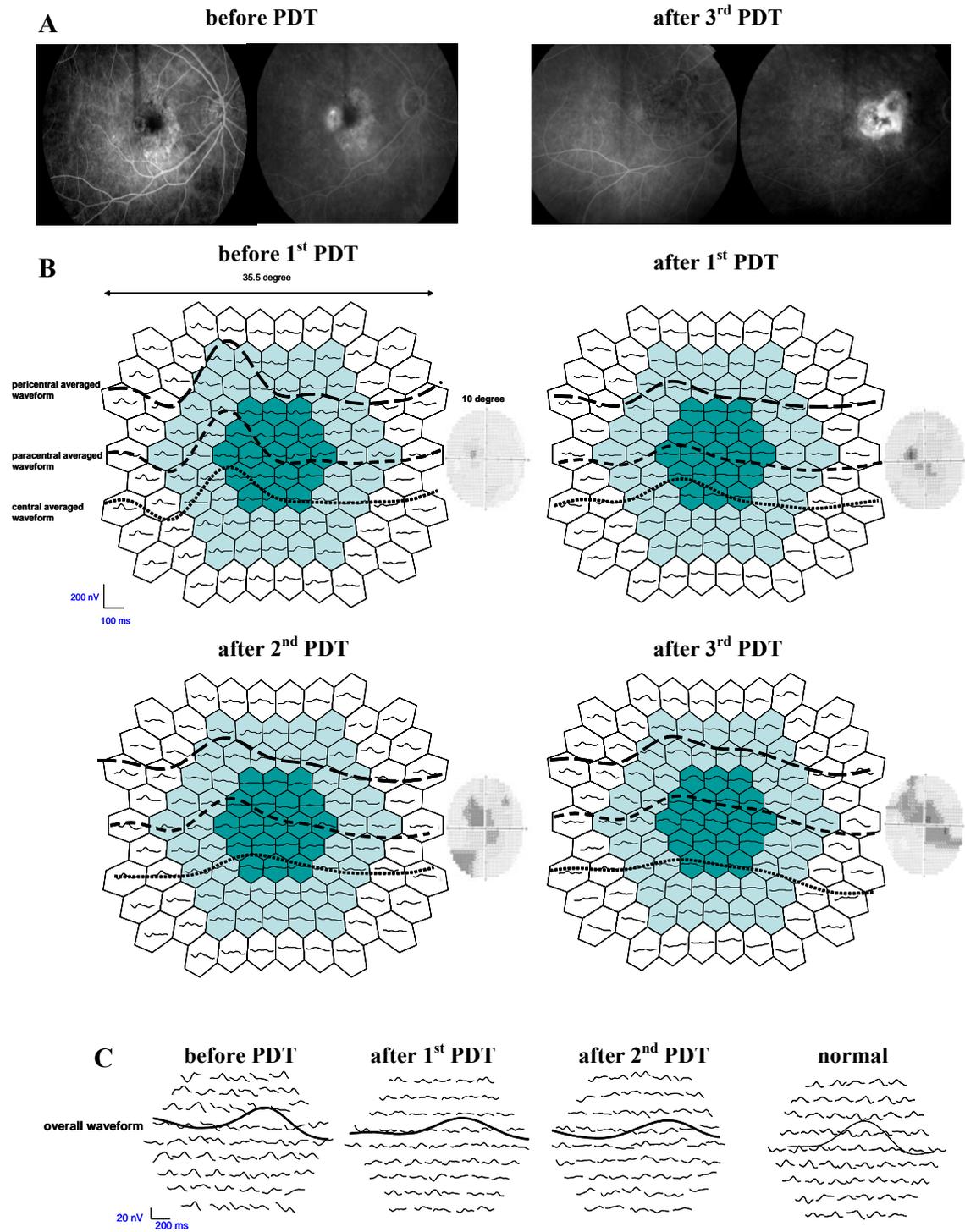


Figure 6.3A-C. (A) The early and late phase FA angiogram of subject 5 before and after 3 PDTs with an increased membrane. (B) Local cone-mediated function as well as central visual fields showed steady deterioration after 3 PDTs. This was significant for all locations for the cone-mediated mfERG a-scales (C). The rod-mediated mfERG responses were impaired after two PDTs but this was not statistically significant (large waveform indicates overall response). The rod-mediated mfERG responses of a healthy subject are shown for comparison on the very right side.

6.4.3 Psychophysical measures

The results of the other vision tests as well as other subject characteristics are outlined in Table 6.1. After three PDT treatments and a follow up period between 4.5 and 6.5 months (mean 5.5 months) stabilisation (fewer than 15 letters loss) (Treatment of Age-Related Macular Degeneration With Photodynamic Therapy (TAP) Group 1999; Treatment of Age-Related Macular Degeneration With Photodynamic Therapy (TAP) Study Group 2001) of visual acuity was achieved in four subjects. One subject (5) showed a significant decrease (greater than 15 letters) in visual acuity. Contrast sensitivity was considered to be stabilized (less than 6 letters change) in two subjects (subjects 1 and 4) based upon the findings of Rubin et al. (2002). Two subjects (2 and 5) lost 15 and 20 letters respectively, and one subject (3) could not perform the Pelli Robson test at baseline. The central visual fields (10 degrees) mean defect (MD) differences are shown in Table 6.1 and show higher MD in 4 subjects (especially subjects 3 and 5) after all treatments.

6.6 DISCUSSION

We were able to monitor cone- and rod-mediated function between multiple PDT treatments using mfERG. We found no deterioration in outer retinal function compared to baseline after three PDTs in four of five subjects for the cone-mediated mfERG and in all eyes for the rod-mediated mfERG despite constant membrane growth. Although there were significant transient impaired amplitudes for the cone- and rod-mediated responses, possibly reflecting choroidal hypoperfusion between the treatments, these returned to baseline values after the second or third treatment. Interestingly a misshapen descending part of the b-waveform of the cone-mediated responses appeared to be more evident after three PDT treatments suggesting post-

receptoral sites, mainly ON and OFF bipolar cells (Hood et al. 1997), to be more affected. We could not find a specific location predilection showing a larger effect on the surrounding para- or pericentral retina except in one subject who showed para- and pericentral improvement of amplitudes.

We found poorly recordable rod-mediated responses at baseline. This is a characteristic feature of AMD at this level of impairment, and based upon histological findings is not surprising. Curcio et al. (1996) demonstrated that the last surviving cells were largely cones in their donor eyes with AMD. We did not expect the rod responses to be comparable to the responses of the cones based upon previous studies (Hood et al. 1998b) and our own experience (Feigl et al. 2004a; Feigl et al. 2004c) showing that local rod-mediated responses gave smaller signals. There were still recordable rod-mediated responses and those did not appear to be more vulnerable than cones to treatment effects after multiple laser applications. However, eliciting rod-mediated responses remains a demanding procedure (Hood et al. 1998b; Feigl et al. 2004a) and might not be suitable for monitoring treatment effects in late ARM in every subject. Recently Jackson et al. (2004) found that the rod-mediated full-field ERG did not detect functional deficits in aging, early ARM or late ARM but they did not rule out the usefulness of a rod-mediated mfERG in detecting changes. We have developed a rod-mediated mfERG procedure in our laboratory and have shown local functional deficits in early ARM subjects (Feigl et al. 2004a; Feigl et al. 2004c). The responses are small and difficult to elicit even in early ARM, and this becomes more evident in late ARM when few rods remain, as illustrated in the 5 subjects investigated here.

Fluctuations of visual performance and recovery after PDT have been previously described (Schmidt-Erfurth 1999). Schmidt-Erfurth and colleagues (1999; 2001; 2004) showed recovery of photoreceptor function after PDT by using scanning laser ophthalmoscope (SLO) microperimetry. They found preservation of central visual fields with significantly smaller mean absolute and relative scotomas in the PDT group compared to a placebo group over a follow up period to two years (Schmidt-Erfurth et al. 2004). They also asserted that repeated PDT applications did not cause additional damage to the treated area, but might further enhance the recovery of macular function (Schmidt-Erfurth 1999). We also found that repeated PDT application did not cause additional impairment, assessed by the cone- and rod-mediated mfERG in most subjects. However, we could not demonstrate enhancement of outer retinal function in most of our subjects after multiple treatments; this may have been due to the fact that they were still undergoing treatment and that our follow up period (mean 5.5 months) was relatively short.

Characteristic short term visual symptoms and clinical findings after PDT treatment, including increased metamorphopsia and intra- and subretinal fluid within the treated area, have been previously reported (Rogers et al. 2002; Schmidt-Erfurth et al. 2002b; Costa et al. 2003; Michels and Schmidt-Erfurth 2003). Those findings have been shown not to correlate with immediate CNV or choriocapillaris closure but possibly with choroidal vascular barrier dysfunction and an acute inflammatory reaction (Michels and Schmidt-Erfurth 2003). Costa et al. (2003) demonstrated a short term effect of PDT with increase in retinal thickness and fragmentation to the retinal pigment epithelium/choriocapillaris reflective band measured with optical coherence tomography (OCT) up to one week after PDT. However, after one month

macular remodelling has been observed using OCT (Rogers et al. 2002; Costa et al. 2003). Similarly short term transient reduction of the cone-mediated mfERG measures and recovery after one month have been shown by Lai et al. (2004). On the other hand Jiang et al. (2003) showed stable measures of outer retinal function 3 and 7 days after treatment compared to pre-treatment values. The reason for differing results from those studies after one treatment might be the high inter-individual variability in choroidal hypoperfusion as Schmidt-Erfurth and Michels (2003) have pointed out. Further, Lai et al. (2004) mainly investigated secondary CNVs (14 out of 17 eyes) rather than CNVs due to AMD (3 eyes). However, our findings of transient impairment of retinal function in some subjects after one month could have reflected the longer term effect on outer retinal function of prolonged choroidal hypoperfusion. MfERG waveforms are thought to be mainly shaped post-receptoral and mainly by ON and OFF bipolar cells (Hood et al. 2002). Thus misshapen waveforms may reflect transient hypoxia. Recently Bui et al. (2003) used the full-field ERG to demonstrate that post-receptoral sites are more susceptible to ischemic insults than are photoreceptors. Additionally Greenstein et al. (2004) described atypical broader first-order shaped waveform in their patients with different retinal diseases and attributed their results to a possible damage to the outer plexiform layer and the bipolar cells.

We think a one month interval for retesting function with the mfERG after PDT could be helpful because the macula has recovered from an acute, possibly inflammatory response (Costa et al. 2003). At this point there might be less masking of treatment effect on choroidal perfusion and secondary effects on outer retinal function.

Schmidt-Erfurth et al. (2002b) also investigated the PDT effects on CNV in a group after one treatment and in another group after three treatments (repeated PDT applications at 4-week intervals) with ICG-A. One important finding of that study was that there was 50% recurrence and persistence and that multiple treatments within short intervals reduced the rate of persistence but not recurrence. Moreover they found that hypofluorescence decreased in size and intensity over time in the single treatment group but enlarged in the multiple treatment group by 25% after the second PDT compared to the area after the first PDT. After the third PDT the decrease of this area was smaller in the multiple treatment regimes than the more intense resolution of hypofluorescence after a single exposure. They suggested that the PDT effect is less intense when choosing shorter retreatment intervals which was the case in our study (on average every eight weeks). They further stated that the next PDT application might impact on an area with persistent vascular occlusion so that less sensitizer and less oxygen are available to finally target the new vessel formation. Our findings of transiently impaired or finally misshapen waveforms might also be explained by this, as recovery of hypoperfusion may be more disturbed in a short retreatment scheme. Recently Stur et al. (ARVO 2004, abstract number 2275: Verteporfin Early Retreatment (VER)- 12 months results of a phase IIIb controlled clinical trial, VER Study group) were unable to show an advantage of early retreatment intervals (every 1.5 months during the first six months) compared to longer retreatment intervals (every 3 months) based on visual acuity outcomes.

Other visual function tests such as visual acuity and contrast sensitivity remained stable in most of our subjects despite the changes in the central visual fields which mainly showed higher MDs. Additionally the central responses of the cone-mediated

mfERG were not always reflected in the visual fields. For example subject 3 (Fig 6.2B) showed a deeper and larger central scotoma after the third treatment but relatively stable central functional results (Table 6.2, central a-scales). This is in concordance with other investigators who have also found variable degrees of agreement between the cone-mediated mfERG and visual fields depending on the disease studied (Greenstein et al. 2000b; Hood and Zhang 2000; Holopigian et al. 2001). Although this has been found only for one subject, the mfERG might give additional information about retinal function that cannot always be measured with the visual field.

Between-subjects variability was to be expected given the small number of subjects in this pilot study. However this could also underline the importance to treat subjects based upon their individual objective functional results, in addition to angiography and visual acuity. Although Jurklies et al. (2002) monitored the natural course of a CNV also in only a small number of subjects (n= 4), they demonstrated that the size of the CNV complex in angiography had only a low to moderate statistical correlation with retinal function as measured with the cone-mediated mfERG. We suggest that in some cases in the future, retreatment decisions might be based upon measures other than angiography and visual acuity such as OCT (Rogers et al. 2002), microperimetry (Schmidt-Erfurth et al. 2004) and the cone-mediated mfERG. Ultimately, a number of tests might be performed before and after each treatment. Although this appears to be a great number of tests to begin with, larger studies and longer follow up might show which of the tests is most sensitive in detecting functional loss. Monitoring treatment effects by objective means might also help save cost. It has been demonstrated that early treatment of AMD with PDT leads to

increased cost effectiveness (Hopley et al. 2004a; Smith et al. 2004). Given that Smith et al. (2004) suggested that treatment of CNV is most beneficial at the stage of “less advanced AMD,” defined as smaller angiographic lesions and better distance visual acuity as the only function measure, it is of great interest to evaluate function as accurately as possible by objective means.

In conclusion our cone-mediated mfERG results show functional stabilisation (stable or improved function) after three PDT treatments in four of five subjects despite membrane growth and deterioration of some subjective vision measures. Whether transiently impaired function reflects choroidal dynamics needs further investigation in a larger sample size. This study suggests the usefulness of the cone-mediated mfERG in monitoring multiple PDT effects on outer retinal function. Objective data on function such as measured with the mfERG might have advantages over subjective observation and measures. However, this study cannot give generalized conclusions as the number of subjects is too small, but it might encourage larger controlled studies to objectively investigate PDT effects by including the cone-mediated mfERG.

6.8 REFERENCES (see Master Reference List)

CHAPTER 7

7.1 Discussion

7.1.1 Summary of results

Given that there is a preferential vulnerability of rods compared to cones in ARM, an objective test to measure rod-mediated function is especially useful. Although there have been guidelines for a rod-mediated mfERG for younger and experienced subjects, those guidelines still needed modification for an older population especially because of stray light (Hood et al. 1998b). In this thesis a mfERG testing protocol was newly developed which demonstrates that the rod-mediated mfERG is impaired in an older group of inexperienced subjects with ARM, compared with an age-matched control group.

The experiments reported in this thesis have shown that both cone- and rod-mediated functions are affected in early ARM and are measurable either by subjective or objective tests. The results of the follow-up study demonstrated that function remained stable after one year in subjects with early ARM but relatively good visual acuity ($\geq 6/12$). In particular, both the averaged and local rod-mediated mfERG latencies were good measures for functional impairment but were not preferentially affected at any specific retinal location in the ARM groups (chapters 4 and 5). However, averaged central and superior retinal latencies were longer compared to peripheral and inferior retinal latencies in both the ARM and control groups possibly reflecting physiological differences. Similar to the latencies of the cone-mediated mfERG (Fortune et al. 1999), rod-mediated latencies showed less variability

compared to amplitudes and were good indicators of early disease (Gerth et al. 2003; Han et al. 2004b).

Besides reduced contrast sensitivity and visual acuity measures (HC- and LC-VA), we found an impairment of the S-cone pathway as previously described in aging and early ARM (Collins 1986; Applegate et al. 1987; Haegerstrom-Portnoy 1988; Sunness et al. 1989a; Eisner et al. 1992) (see section 2.1.8.2). While S-cone sensitivity loss occurs as a natural part of the aging process, just as the lens of the eye absorbs more light at short wavelengths, there is strong evidence that sensitivity loss is also due to retinal causes (Greenstein et al. 1989b; Coile and Baker 1992; Curcio et al. 1993; Suzuki et al. 1998). Furthermore, impaired S-cone sensitivity has been demonstrated objectively in healthy pseudophakes with the full-field ERG during aging (Suzuki et al. 1998). The findings in this thesis give evidence for retinal causes for S-cone sensitivity loss because a lens grading system was used (Age-Related Eye Disease Study Research Group 2001b) and subjects with signs of cataract were excluded.

Finally, transiently impaired amplitudes found after multiple PDT treatments in late ARM possibly reflected choroidal hypoperfusion and suggest that the mfERG is useful in monitoring function. Whether the cone- and rod-mediated mfERG could become an additional tool for retreatment decisions still needs to be investigated in a larger sample including a control group. However, the pilot study results are in accordance with previous findings after one PDT (Palmowski et al. 2002) and suggest that there is no additional impairment either of the treated or of the healthy surrounding retina after multiple PDTs.

The four hypotheses established at the beginning of the study (chapter 1) were supported in part. The first hypothesis that the cone-mediated mfERG would be affected prior to psychophysical measures and funduscopy for detecting early ARM was not verified (chapter 3). However, the rod-mediated mfERG detected functional impairment before the cone-mediated mfERG (chapter 4) supporting hypothesis 2, but not before commonly used subjective function tests (chapter 5). The findings that the rod-mediated mfERG function did not indicate significant progression in ARM after one year, as did none of the vision tests used in these experiments, did not support the third hypothesis (chapter 5). Finally the fourth hypothesis was verified as no significant further deterioration of outer retinal function as measured by cone- and rod-mediated mfERG was found after multiple PDT treatments (chapter 6).

The results have raised several questions which are addressed in the following sections. Firstly, reasons for the contradictory findings of the cone-mediated mfERG in this thesis compared to other studies are discussed in section 7.1.2. The preferential S-cone and rod vulnerability is discussed (7.1.3) and based on the experimental findings a new ARM model is proposed (7.1.4 and 7.1.5). Finally use of the mfERG in early and late ARM is discussed particularly with respect to its role in documenting progression of ARM and monitoring treatments (7.1.6).

7.1.2 What does the conventional cone-mediated mfERG measure?

It is known that the human first-order cone-mediated mfERG response is mainly shaped by ON and OFF bipolar cells and that there is only a small photoreceptor and inner cell contribution (Hood et al. 2002) (see section 2.2.2). However, different protocols can reveal different cell contributions to the mfERG waveform response.

Although there are guidelines for recording a conventional cone-mediated mfERG (Marmor et al. 2003) these still permit a lot of “freedom”. Given that filter bandwidth, contrast, luminance, electrodes and analysis methods influence results to a great extent (see section 2.2.1), it is not surprising that cellular contributions to the mfERG waveform can vary within an ocular condition even if the ISCEV guidelines are used.

The choice of a high pass filter can be crucial in detecting a specific waveform shape (e.g. negative ERG) which might be missed (if greater than 5 Hz) even though the recommended high pass filter (between 3-10 Hz) is used (Keating et al. 1997; Keating et al. 2000). It is known that reduced contrast or luminance conditions can make inner retinal contributions in the waveform more prominent (Hood 2000). Hood et al. (2000) demonstrated that a mean luminance of 100 cd/m² (most commonly used) detects nasotemporal asymmetry, but this becomes more evident at lower luminances. Therefore although still within the recommended ISCEV range (mean luminance about 50-100 cd/m²) the inner retinal contributions might contribute more or less to the response signal. Most of the major studies with the cone-mediated mfERG in early ARM have used a mean luminance of 100 cd/m² (Huang et al. 2000; Palmowski et al. 2001; Gerth et al. 2003). Unfortunately, Li et al. (2001) who also showed impaired results in early ARM, did not specify the luminance conditions used in their study. However, a number of studies also using lower mean screen luminance conditions have shown reliable results in different diseases (Kretschmann et al. 1998a; Kretschmann et al. 1998c; Chan and Brown 2000; Kretschmann et al. 2000; Scholl et al. 2002). Given that a mean luminance of 50 cd/m² was used for the cone-mediated mfERG in this study, different outcomes

compared to other studies using 100cd/m^2 are to be expected. It is important to describe luminance conditions and consider these when comparing different study results investigating the same disease.

As discussed in chapter 3 there might be several other reasons for the normal cone-mediated mfERG in the ARM group in this study, compared to impaired results found in other studies (Huang et al. 2000; Li et al. 2001; Palmowski et al. 2001; Gerth et al. 2003). These reasons might include varying numbers of subjects, groups not age-matched and/or different analysis methods in different studies. For example, one study included both eyes (Gerth et al. 2003) in their analyses which is regarded as statistically not correct as the two eyes are not independent and most measures are highly correlated. However, given that there is evidence of different incidence rates of right and left eyes in ARM (Wang et al. 2003a; Wang et al. 2004), one might argue that they can be considered reasonably independent. Different averaging methods (averaging concentric rings, hemifields or central/overall ratios) and inconsistent fundus grading have been used previously which might make comparisons between the studies difficult. Even using the same fundus grading system can result in great variability (Scholl et al. 2003). Scholl et al. (2003) showed that the variability of ARM features can be partly explained by intra- and inter-observer variability.

Additionally the non-significant outcome for the cone-mediated mfERG might have been due to the subject selection criteria.(Chen et al. 2004). Most recently Chen et al. (2004) also could not find a significant difference of the cone-mediated mfERG responses in their early ARM subjects compared to a control group and suggested

that their subjects were less advanced compared to the ARM subjects in other studies. The subjects were mainly recruited from an optometry clinic as opposed to ophthalmological practices. Usually the latter group has more progressed ARM signs compared to subjects from an optometry clinic who are likely to have very early signs of ARM when undergoing routine clinical examination.

There might be also the likelihood of non-uniform progression of ARM among subjects, despite typical fundusoscopic changes. Assuming a genetic predisposition in ARM (De Jong et al. 2001; Bird 2003) there are probably different phenotypes resulting in varying functional impairment. Given that function is poorly reflected funduscopically (Sunness et al. 1988; Collins and Brown 1989b; Sunness et al. 1989a; Cheng and Vingrys 1992; Steinmetz et al. 1993; Tolentino et al. 1994) different studies have probably investigated different phenotypes and “stages” of ARM not reflected in the fundusoscopic changes. Moreover, there is a decrease in photopigment, a misalignment, broadening and displacement of the photoreceptors which may or may not alter visual processing in early ARM (Burns et al. 1991; Coile and Baker 1992; Curcio et al. 1996; Elsner and Burns 2002). There is obviously a fine line between results that can be considered as due to normal aging and a beginning impaired function. Variable results might be the outcome when photoreceptors operate at their limits or are compensated in function by the remaining photoreceptors (Curcio et al. 1996).

The conventional mfERG testing protocol has been shown to poorly correlate with other function tests in some ARM studies (see also 2.2.1.9) but these included mainly visual acuity and visual fields (Yoshii et al. 1998; Greenstein et al. 2000b; Hood and

Zhang 2000; Holopigian et al. 2001; Jurklies et al. 2002; R  ther et al. 2003). Previously, the mfERG has been considered to be an objective test of the visual field. Recent studies indicate differences between these two tests even when matching stimulus parameters (Seiple et al. 2002). Seiple et al. (2002) speculated that results might depend on how a retinal disease affects sensitivity as a function of adaptation. There were no significant correlations between the cone-mediated mfERG and the visual field and other central vision measures in this thesis, but significant correlations were found between the cone-mediated mfERG and the desaturated Panel D-15. Given that the S-cone sensitivity loss is mainly attributed to involvement of post-receptoral sites (Greenstein et al. 1989b; Suzuki et al. 1998) and post-receptor contributions become more evident under lower luminance conditions (Hood 2000), the luminance levels used in this study (mean 50cd/m²) might have captured an S-cone pathway deficit. This is highlighted by studies showing that disease-produced sensitivity losses are dependent on adaptation levels (Sandberg and Berson 1977; Greenstein et al. 1989a; Yeh et al. 1989). It has been suggested that the detection of a selective loss of the S-cone pathway may depend on the choice of adapting intensities and the effect of background luminance (Greenstein et al. 1989a; Yeh et al. 1989).

In contrast to the subjective vision measures such as HC-, LC-VA, low luminance VA, contrast sensitivity and colour vision, the cone-mediated mfERG did not detect function loss in early ARM. This is probably due to methodological differences between the mfERG and the psychophysical tests, e.g differing luminance, stimulus and background conditions, or the differing mechanisms of response generation, adaptation level and response measure (Seiple et al. 2002). The mfERG response

reflects the summed activity of a stimulus consecutively presented in a fast flickering (dynamic) mode from many local areas measuring suprathreshold activity. Conversely, the psychophysical tests used in this thesis were foveal, static tests of threshold function, thus measuring different aspects of vision. The conventional (61 or 103 hexagon) mfERG uses a lower spatial resolution (central hexagon $> 1^\circ$) and the central hexagon usually covers a larger area than the foveal response measured with psychophysical measures. Phipps et al. (2003) showed that visual acuity correlated significantly with steady state threshold tests such as spatial and temporal contrast sensitivity but not with tests measuring dynamic thresholds.

7.1.3 Why are S-cone and rod pathways affected in early ARM?

The macula is apparent by about 11-13 weeks gestation (Marshall 1987). Although the precursors of both rods and cones appear to be at a similar level of development at 14-15 weeks of gestation, rods undergo morphological maturation earlier than L- and M-cones (Marshall 1987). Similarly, S-cones have been shown to develop very early throughout gestation. They show adult-like distribution at the 20th week of gestation, even before the foveal depression has developed. After birth, psychophysical measures demonstrate that infants as young as five weeks can discriminate tritan pairs (Narayanan and Wadhwa 1998). On the other hand, L- and M-cones still undergo modifications with centralwards migration, increase in foveal packing density, elongation and thinning up to four years of age (Varner et al. 1985). Adult humans have a distinct but variable central zone about 100 μ m wide that lacks S-cones and is surrounded by a ring in which the S-cone density is 8% (Yuodelis and Hendrickson 1985). This S cone-free zone is detectable at fetal week 15.5, shortly after S-opsin is expressed. It has been suggested that foveal S-cones migrate

parafoveally, or switch phenotype by changing from S opsin to L/M opsin (Bumsted and Hendrickson 1999).

It could be hypothesized that due to similar embryogenic development, rods and S-cones might be linked or be vulnerable to metabolic insults in a similar pattern. Indeed, rod involvement with S-cone pathways has been suggested (Bumsted and Hendrickson 1999). For example, under dark-adapted conditions, rod influence produces a light-level-dependent increase in errors on the Farnsworth-Munsell 100-hue test (FM 100). Rods impair discrimination mediated by S-cone pathways, which at moderate levels of illumination differentially elevate tritan errors on the FM 100 (Knight et al. 1998). This has been shown to be independent of pupil size or mismatching due to scotopic brightness cues competing with weak S-cone mediated hue signals (Knight et al. 1998). The authors concluded a direct effect of rod signals on S-cone mediated chromatic discrimination.

Both S-cone pathways and rods have been shown to be sensitive to hypoxia. In several ischemic diseases, such as diabetes and open angle glaucoma, selective losses of S-cones have been demonstrated (Greenstein et al. 1987; Greenstein et al. 1989b; Yamamoto et al. 1996). It has been shown that hypoxia causes a tritan deficiency (Smith et al. 1976). Additionally rods require larger amounts of oxygen than cones, which can lead to the deterioration of diabetic retinopathy as rods impose additional hypoxia as an oxygen sink especially in the dark adapted state (Arden et al. 1998). Furthermore, impaired oscillatory potentials which are thought to mainly depend on rod-input (Wu and Sutter 1995) have been demonstrated in diabetes and are thought to reflect the ischemic condition (Kurtenbach et al. 2000). Other evidence of the

similar vulnerability of S-cones and rods is the drastic reduction of rods and S-cones compared to L- and M-cones in progressive ARM (Curcio et al. 1996). Hypoxia might be the major insult in ARM as suggested by findings of reduced choroidal blood flow (Remulla et al. 1995; Grunwald et al. 1998; Grunwald et al. 2005) and age-related changes in Bruch's membrane (Pauleikhoff et al. 1990a; Chen et al. 1992) which possibly lead to a decrease in diffusion of oxygen to the neurosensory retina (Grunwald et al. 2005). Similar risk factors such as smoking and high plasma cholesterol in ARM and cardiovascular diseases suggest a common ischemic pathogenesis (The Eye Disease Case-Control Study Group 1992; Klein et al. 1998; Evans 2000; Klein et al. 2003b).

Apart from a possible similar predisposition for S-cones and rods to be affected in ARM, other factors might also play a role that both pathways are involved. For example the S-cone pathways have been always considered to be more vulnerable than other cone pathways in retinal diseases (Marre and Marre 1972; Sandberg and Berson 1977; Pokorny et al. 1979; Young 1982). Based upon "Koellner's rule" subjects with diseases of the retina tend to have disordered colour vision characterized by a preferential loss of the ability to discriminate blue from yellow hues. Acquired colour vision deficiencies that result from retinal diseases have been demonstrated to be tritan-like defects in early stages but subsequently change to red and green deficiencies (Hart 1987). For example, it has been shown that diabetes can produce a reduction in the sensitivity of the S-cone pathway by over a factor of 10, with little loss of sensitivity of other cone pathways (Marre and Marre 1972; Greenstein et al. 1989b). Whether S-cone pathways are more vulnerable than the L- and M-cones due to a more limited response range, have a lower physiological

reserve (“fragile receptor hypothesis” (Hood and Greenstein 1988)) or are affected due the scarcity of S-cones have been the subject of considerable discussions (Hood et al. 1984; Hood and Greenstein 1988). It is known that S-cones are sparse and signals from multiple blue cones converge onto smaller numbers of ganglion cells than L and M cones (Hood et al. 1984). Similarly, ganglion cells conducting blue/yellow contrast information are very few and their receptive fields are sparsely distributed and have less overlap. Additionally there is strong evidence that the path taken from photoreceptor to the LGN by S-cone signals is separate from that taken by M- and L-cone signals (Dacey and Lee 1994; Hendry and Calkins 1998). However, scarcity does not predict differential vulnerability as shown by Hood et al. (1984) in early studies. They assessed the relative response ranges of the S- and L-cones by using the probe-flash paradigm and equally adapted conditions for both pathways. Their findings showed a limited response range of the S-cone pathway compared to the L-cone pathway. Hood et al. (1984) speculated that scarcity does not predict differential vulnerability but might contribute to the functionally restricted response.

There might be a number of possible influences on the rods to make them preferentially vulnerable in early ARM besides the vitamin A deficiency hypothesis of Curcio et al. (2000). They suggested reduced translocation of the retinoids from the blood across Bruch’s membrane due to accumulated cell debris (Curcio et al. 2000). However, many risk factors such as ocular pigmentation and melanin concentration levels, the carotenoid (lutein and zeaxanthin) status as well as gender and hormonal influences might be also linked to a preferential effect on the rods (Weiter et al. 1985; Klein et al. 1997b; Smith et al. 1997; Frank et al. 2000; Klein et

al. 2000; Rapp et al. 2000; Snow et al. 2002). In addition recent human and animal studies in ARM eyes have found loss of photoreceptors, preferentially affecting the rods, RPE cells and inner nuclear layer cells by apoptosis (Hinton et al. 1998; Del Priore et al. 2002; Dunaief et al. 2002; Gordon et al. 2002).

Strong correlations between melanin content and rod numbers have been shown in recent animal studies (Donatien and Jeffery 2002). In normal human eyes melanin is less concentrated in the macular-perimacular region but its concentration is highest in far peripheral areas, and parallels the distribution of rods (Schmidt and Peisch 1985). Melanin is an important photoprotector and antioxidant as it absorbs light directly and serves as a scavenger of light-induced free radicals (Weiter et al. 1986; Boulton and Dayhaw-Barker 2001). Correlations between ARM and decreased ocular pigmentation have been reported (Frank et al. 2000; Donatien and Jeffery 2002). Furthermore histological studies of the RPE in normal postmortem donor eyes show about a 35% decline in melanin granules in the macular RPE after the age of 40 years (Feeney-Burns et al. 1984; Schmidt and Peisch 1985; Weiter et al. 1986; Boulton and Dayhaw-Barker 2001) which is in close accordance with the 30% decrease in rod numbers during normal aging reported by Curcio et al. (1993) and increased lipofuscin accumulation in RPE cells with age (Feeney-Burns et al. 1984).

Lutein and zeaxanthin which are important antioxidants have been shown to exist in rod outer segments (Rapp et al. 2000). Given that reduced levels of lutein and zeaxanthin have been reported in ARM (Mares-Perlman et al. 2001; Gale et al. 2003), the rods might be preferentially affected because of reduced levels of protection.

Another reason that rods are preferentially vulnerable might be associated with oestrogen. Women aged 75 years or older have approximately twice the risk of men of developing ARM (The Beaver Dam Eye Study 2002). Although there are studies to the contrary relating to menopause and ARM (Klein et al. 2000), beneficial effects of endogenous oestrogen or of hormone replacement therapy in reducing the risk of advanced types of ARM have been shown (Klein et al. 1997b; Smith et al. 1997; Snow et al. 2002). Snow et al. (2002) showed that an older age of menarche was associated with increased risks of advanced ARM. It is known that oestrogen regulates the enzyme cathepsin D which is highly expressed in the RPE lysosomes and is important for its phagocytosis and digestive capacity (Snow et al. 2002). While cathepsin D exists in numerous cells in the body, its highest concentration is in the RPE cells (Adler and Martin 1983) and it shows a rhodopsin specific enzymatic activity (Regan et al. 1980; Rakoczy et al. 1996). Experimental in-vitro and in-vivo studies in mice have shown that an induced impairment of cathepsin D causes an accelerated accumulation of rod outer segment debris due to a breakdown of the function of one of the major RPE enzymes (Rakoczy et al. 2000).

7.1.4 Models of early ARM

A large number of psychophysical vision tests have shown impairment of cone- and rod-mediated function in early ARM (Brown and Kitchin 1983; Brown et al. 1986a; Brown and Lovie-Kitchin 1987b; Collins and Brown 1989b; Haegerstrom-Portnoy and Brown 1989; Cheng and Vingrys 1992; Eisner et al. 1992; Midea et al. 1997; Owsley et al. 2000; Owsley et al. 2001; Phipps et al. 2003) (see section 2.1.8.2).

It is still not clear whether functional deficits in ARM measured with various vision tests are primarily caused by structural abnormalities of the photoreceptor (Smith et al. 1988; Elsner and Burns 2002; Phipps et al. 2003), by post-receptoral damage (Phipps et al. 2003) or by damage to other tissues involved in ARM such as the RPE/Bruch's membrane complex (Phipps et al. 2003). In contrast to a structural abnormality of the photoreceptor (abnormal orientation, misshaping, photoreceptor loss) which might result in decreased photopigment and photosensitivity (Elsner and Burns 2002), the "kinetic hypothesis" suggests slowed regeneration of photopigment due to slowed transfer through Bruch's membrane (Curcio et al. 2000; Owsley et al. 2001; Haimovici et al. 2002; Phipps et al. 2003). Thus the kinetic hypothesis predicts that cones and rods have abnormal altered recovery dynamics caused by dysfunction in the photopigment regenerative capacity (Eisner et al. 1992; Steinmetz et al. 1993; Owsley et al. 2001; Phipps et al. 2003). This model would also suggest that the amount of abnormal deposits and thus drusen correlates with poorer recovery dynamics. In fact, the contrary has been shown in a number of studies and poor correlations have been reported between drusen and kinetic measures such as dark-adaptation or glare recovery (Smiddy and Fine 1984; Collins and Brown 1989b; Eisner et al. 1991; Cheng and Vingrys 1992).

Recently Elsner and Burns (2002) hypothesized that decreased photosensitivity did not imply primary damage to Bruch's membrane but might be due to alteration in the microenvironment of the photoreceptor. They used colour match/illuminance techniques and demonstrated that decreased photosensitivity of the cone photopigment was uncorrelated to slowed regeneration kinetics in early ARM (Elsner and Burns 2002). This suggests that the dynamic model does not explain all

functional deficits in ARM and that there must be other factors possibly relating to microenvironmental alterations such as, for example, hypoxia. Collins and Brown (1989a; 1989b) hypothesised that significant delay of glare recovery time was due to hypoxia. They explained their findings with abnormal adaptation mechanisms at the photoreceptor level. Given that hypoxia shows more effect on post-receptoral sites (Cringle et al. 2002; Bui et al. 2003), alteration of post-receptoral pathways and modulatory cells throughout the retina might be also possible.

Another model, the so-called “hemodynamic model”, has been proposed by Friedman et al. (1995) and is based upon impaired choroidal perfusion. They measured a higher pulsatility of the central retinal artery and the nasal/temporal short ciliary arteries compared to an age-matched control group with colour Doppler imaging in subjects with early and late ARM. Friedman et al. (1995) hypothesised that a progressive decrease in the compliance of the sclera and choroidal vessels is initiated by the deposition of lipid in the sclera and Bruch’s membrane. This might result in a higher intravascular pressure with decreased perfusion. In contrast to the kinetic model where lipids are thought to slow passage of material through Bruch’s membrane, this model suggests that deposition of lipids increases the resistance of the choroidal vessels. The RPE is hypothesized then to decompensate due to its decreased capacity to pump fluid and metabolites against unfavourable hydrostatic and osmotic pressures. Based upon these two models and the thesis results, a “hypoxia post-receptoral” ARM model is proposed (see next section).

7.1.5 Hypoxia post-receptor model

Given that 65% of the ARM subjects compared to 25% of the control subjects in this thesis had at least one of the risk factors such as hypertension or high blood cholesterol for developing ischemic diseases or had another disease causing chronic hypoxic conditions such as asthma (Klein et al. 2003), chronic ocular hypoxia might be the primary insult. Most recently Grunwald et al. (2005) have suggested that reduced choroidal blood flow could lead to hypoxia. Together with other authors (Linsenmeier and Padnick-Silver 2000) they have hypothesised that thickening of the RPE/Bruch's membrane complex would increase the distance that oxygen must travel from the choriocapillaris to the retina and that this would reduce the availability of oxygen in the outer retina. Grunwald et al. (2005) demonstrated that eyes with advanced ARM fundus features such as drusen and RPE abnormalities tended to show more pronounced decrease in choroidal blood flow. Johnson et al. (2005) showed in histopathological studies in a diabetic, hypertensive monkey that choroidal dysfunction was associated with age-related macular degeneration-like changes in Bruch's membrane. Several studies show that the post-receptor driven bipolar cell response is lost after only minutes of ischemia whereas photoreceptor function persists longer (Winkler 1981; Winkler 1983; Ames et al. 1992; Bui et al. 2003). S-cone pathway sensitivity loss in other ischemic diseases or aging is thought to be mainly generated post-receptorally (Holopigian et al. 1997a; Suzuki et al. 1998). In addition, the rod-mediated mfERG response is mainly generated post-receptorally (Hood et al. 1998b). Based upon the fact that these photoreceptors and their pathways are thought to be more vulnerable to hypoxia (Smith et al. 1976; Greenstein et al. 1989b) the model described below (Fig. 7.1 A-E) might be used to explain most of the findings reported here.

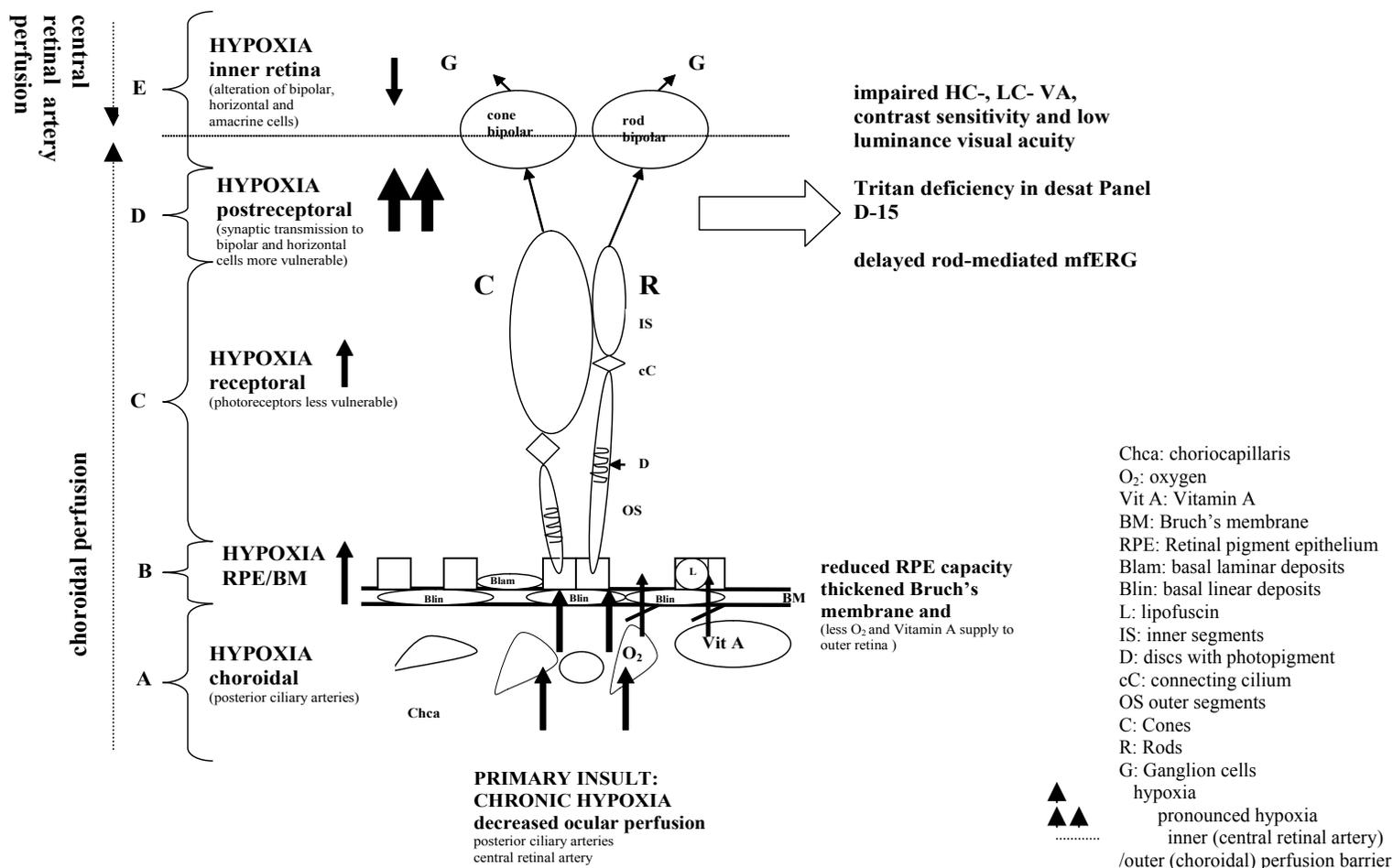


Fig. 7.1A-E. The schematic "hypoxia-post-receptor model" in early ARM

We suggest that chronic ocular hypoxia might be the primary insult in ARM. There is a significant relationship between higher systolic blood pressure and the development and progression of ARM (Klein et al. 2003b). Increased blood pressure and atherosclerosis and their effects on the ocular circulation and Bruch's membrane have been hypothesised to increase the risk for developing ARM (Kornzweig 1977; Pauleikhoff et al. 1990a). Vascular deficits have been identified in early and late ARM using fluorescein and indocyanine green angiography, laser doppler flowmetry and colour doppler imaging (Pauleikhoff et al. 1990a; Chen et al. 1992; Grunwald et al. 1998; Ciulla et al. 1999; Pauleikhoff et al. 1999; Grunwald et al. 2005). Reduced choroidal blood flow and prolonged choroidal filling in FFA and ICG-A have been demonstrated in ARM (Chen et al. 1992; Friedman et al. 1995; Zhao et al. 1995; Ciulla et al. 1999; Pauleikhoff et al. 1999; Ciulla et al. 2001b; Grunwald et al. 2005). Colour doppler imaging measurements of the choroidal posterior ciliary arteries as well as the central artery demonstrate reduced blood flow in early and late ARM subjects compared to an age-matched control group (Friedman et al. 1995; Ciulla et al. 1999) suggesting chronic ocular hypoxia in the outer and inner retina. While perfusion abnormality in ARM has been thought to reflect an attenuated choriocapillaris with narrowing lumen (Kornzweig 1977) and thinning of the choroid as demonstrated histologically (Sarks 1976), increased resistance of the choroid to blood flow by deposition of lipids in the sclera and in Bruch's membrane has been also hypothesised ("hemodynamic model") (Friedman et al. 1995).

The macular choroid is most vulnerable to ischemic disorders because of the numerous "watershed" zones meeting there (Hayreh and Gartner 1990). Subsequently, chronic choroidal hypoxia (Fig 7.1.A) might lead to impaired function

of the RPE with decreased degradation activity of photoreceptor disc membranes, antioxidant capacity and more deposition of abnormal debris, the basal linear (Blin) and basal laminar (Blam) deposits (Fig 7.1.B). A thickened Bruch's membrane might not only lead to reduced diffusion of oxygen (Grunwald et al. 2005) and thus more hypoxia but also to an impaired transport capacity (Starita et al. 1995) of important metabolites to the retinoid cycle (such as Vitamin A, trans-ROL) (Fig 2.6). Thus the photoreceptor circulation current is altered as synthesis of 11-cis retinal (cis-RAL) is slowed in the RPE as is proposed in the "kinetic model". It is likely that the photoreceptors are more resistant to hypoxic insults than post-receptoral sites due to their proximity to metabolic reserves available in the RPE and the choriocapillaris (Cringle et al. 2002) which is reflected in less functional alteration (Fig 7.1.C).

The choroid supplies the overlying retina to a depth of 130 μ including not only the RPE and adjacent outer retina but also the outer part of the inner nuclear (bipolar cell) layer (Hayreh and Gartner 1990). Hypoxia might have more of an effect at the post-receptoral levels (Fig 7.1.D) as photoreceptors act like an oxygen sink. Experiments in rats have demonstrated that oxygen uptake in the inner retina exceeds that of the outer retina (Cringle et al. 2002). Yu and Cringle (2001) suggested that there is a high rate of oxygen consumption in the outer and inner plexiform layers where there is high synaptic activity between photoreceptors and bipolar and horizontal cells and between bipolar, ganglion and amacrine cells, respectively. Electrophysiological evidence of preferential post-receptoral susceptibility to hypoxia has been given by Bui et al. (2003) most recently. They have demonstrated a strong correlation between full-field electrophysiological and neurochemical changes following an ischemic insult. Decay in phototransduction correlated strongly with

extracellular accumulation of amino acids and heightened glutamate oxidation, typical for post-receptor damage. There was a post-ischemic b-wave loss whereas the a-wave and thus receptor responses seemed relatively unaffected.

Bui et al. (2003), Grozdanic et al. (2003) and Shibuki et al. (2002) have investigated acute hypoxia with electrophysiological measures such as the full field ERG or flicker ERG. It is known that acute ischemic events cause massive damage to the outer and inner retina histopathologically (Grozdanic et al. 2003; Johnson et al. 2005), which is indicated electrophysiologically in a b-wave amplitude loss reflecting postreceptor alteration and a reduction in oscillatory potentials reflecting inner plexiform layer cell alterations (Ogden 1973; Coleman et al. 1990; Block and Schwarz 1998; Grozdanic et al. 2003; Johnson et al. 2005). However, delayed peak implicit times have also been reported (Bui et al. 2003; Grozdanic et al. 2003). Chronic hypoxia might induce impaired synaptic transmission post-receptorally resulting in delayed rod-mediated latencies before amplitudes are involved (Fig 7.1.D). It has been suggested that selective post-receptor loss reflects synaptic signalling between photoreceptors and bipolar cells (Bui et al. 2003). This would be also in accordance with Hood et al.'s (2000) findings for the cone-mediated mfERG that altered synaptic transmission in the outer plexiform layer (OPL) (see also section 2.2.2.2) can cause a latency delay with less effect on amplitude. In fact in diabetes, it has been shown that latencies are most sensitive in detecting hypoxic conditions before amplitudes are affected and even before fundus changes are evident (Han et al. 2004b).

Operating at lower oxygen levels could imply that post-receptor sites are less able to meet metabolic challenges, (as required, for example in responding to a flickering stimulus (Kiryu et al. 1995; Falsini et al. 2003)), than receptor sites. In primate retinas it has been shown that the blood flow in retinal arteries increased by 30% in response to a greater metabolic challenge from monochromatic light flicker (Kiryu et al. 1995). Falsini et al. (2002) have found that increased blood flow in the optic nerve correlated with a greater neural activity, as measured with the flicker ERG in humans. They showed reduced retinal flicker sensitivity with the focal ERG in early ARM (Falsini et al. 2003). Recently Phipps et al. (2004) proposed that flicker thresholds are more adversely affected than steady state thresholds in early ARM. They proposed a metabolic challenge model, suggesting that photoreceptors are close to their functional limits because the higher metabolic demands and blood flow which are required by flicker stimuli cannot be supplied in ARM.

Given that the mfERG uses flickering stimuli, a greater metabolic challenge for the rods could explain the rod-mediated mfERG results. The reason why the cone-mediated mfERG was not significantly affected in early ARM in this study is hypothesised to be the L- and M-cone pathways greater metabolic reserve and less hypoxia vulnerability compared to the rod-mediated responses and possibly a very early stage of the disease. It is hypothesised that a desaturated colour vision tests is a metabolic challenge to the S-cone pathways; their limited range of response (Hood et al. 1984) and greater vulnerability to hypoxia might cause greater functional alteration compared to L-and M-cones.

Although a primary receptor origin of damage in early ARM cannot be excluded based on the other impaired results (HC-, LC- low luminance visual acuity and contrast sensitivity) (chapters 3 and 5), these losses might be also mainly due to damage at post-receptor sites (Fig 7.1 D-E). Loss of contrast sensitivity has been thought to relate to decreased efficiency in lateral inhibitory mechanisms which are mediated by horizontal and amacrine cells (Brown and Garner 1983; Brown and Lovie-Kitchin 1987a). Brown and Garner (1983) and Brown and Lovie-Kitchin (1987a) showed that adaptation mechanisms which optimize contrast detection are compromised in early ARM. The contrast sensitivity function was mainly disrupted at photopic and mesopic luminances and at low (between 0.5 and 1.0 c/deg) and higher (between 10 and 16 c/deg) spatial frequencies in ARM subjects compared to a control group (Brown and Garner 1983). They speculated that this was due to a decrease in signal/noise ratio because of less lateral inhibition of changes in spatial summation (Brown and Garner 1983). Reduced ocular (choroidal and inner retinal) blood flow might not only alter synaptic transmission between photoreceptors/bipolars and the dendritic ramifications of horizontal and amacrine cells in the outer plexiform layer but might also alter cell metabolism in the inner retina (Fig 7.1.E).

Although the results in late ARM are based upon observations in only a few subjects, the hypoxic post-receptor model might also explain the transiently impaired amplitudes and increasingly misshapen waveforms affecting the descending part of the b-wave of the cone-mediated mfERG after several PDTs in late ARM. Given that there is evidence of prolonged choroidal hypoperfusion after PDT (Schmidt-Erfurth

and Michels 2003), cumulative effects of PDTs with pronounced hypoxia might have preferentially affected ON and OFF bipolar cells.

Assuming ARM is initiated by chronic hypoxia it still remains to be determined what causes this condition. However, this model would explain differing functional test results depending on the degree of hypoxia. This model does not exclude photoreceptor damage as suggested by some studies but speculates that this would happen later in the course of the disease. Thus the subjects involved in this study might have had very early ARM compared to other studies. Similar to previous reports (Hart and Burde 1983; Brown and Lovie-Kitchin 1987a; Swann and Lovie-Kitchin 1991; Curcio et al. 1996), the model proposes that in the early course of the condition, functional disturbances occur eccentric to the fovea first which would underline findings of delayed rod-mediated responses and S-cone pathway deficiency which are located parafoveally.

7.1.6 The mfERG and its role in early and late ARM

One of the greatest risk factors of ARM is age. With an aging population the disease is expected to become epidemic over time. The costs of this condition to the health system in Australia have been calculated to exceed AUD\$240 million on average per year (Hopley et al. 2004a). Although studies providing similar data from the USA and Europe have not been performed, it can be expected that the cost will be much higher. Meads and Hyde (2003) have reported a review of published estimates of the costs of blindness including AMD in several countries most recently.

There are limited options for treatment and prevention of progression is one of the most important goals in ARM. Cost effectiveness of preventive strategies and treatments such as PDT have become a major issue in public health systems and the basis of recent research (Hopley et al. 2004a; Hopley et al. 2004b; Smith et al. 2004). The costs of screening for, and the prophylactic treatment of, early ARM with antioxidants and high dosages of zinc have been calculated to be effective, and to save enormous expenses when compared with no screening or treatment (Hopley et al. 2004b). The cost effectiveness of treatment with PDT has been suggested to be reasonable in subjects with visual acuity of 6/12 at baseline and small, predominantly classic CNV (Hopley et al. 2004a; Smith et al. 2004). Evaluation of diagnostic methods to estimate disease progression and/or documenting treatment effects such as funduscopy and angiography is essential, but these methods are not sufficient. Other objective tools, such as the mfERG for detecting and monitoring retinal function in ARM may be advantageous.

Based upon the experiments of this thesis the cone- and rod-mediated mfERG are useful for documenting the functional state of the retina in early and late ARM (chapters 3, 4, 5 and 6). Early ARM progresses slowly based on funduscopy appearance (van Leeuwen et al. 2003b) which has been confirmed functionally as no further progression in subjective and objective vision measures was evident after a follow-up of one year (chapter 5). These findings might help to save costs in future as ophthalmic control of patients with bilateral early ARM might not be necessary before an interval of one year.

An objective documentation of retinal function after PDT is required to understand the effects on overall functional integrity. It is still not clear what functional consequences several PDTs have on outer retinal function. A prolonged choroidal hypoperfusion with deeper relative scotomata as well as poorer visual outcome after several PDTs compared to one PDT treatment have been described with subjective measures (Schmidt-Erfurth and Michels 2003; Schmidt-Erfurth et al. 2004). Similarly it is not clear how multiple treatments affect the surrounding healthy retina as the size of the laser spot used in PDT treatment exceeds the affected area. The pilot data of this study suggest that objective function does not further deteriorate and that the surrounding retina is not significantly affected.

The appropriate number of PDT retreatments remains controversial. The results of the experiments reported in chapter 6 indicate that the cone- and rod-mediated mfERG can provide an objective measure of function after PDT. This could help in the retreatment decision. However, the weakness of this pilot study is that it lacks a control group and the findings were from only a small group of subjects (n=5). Nonetheless, iteration starts with pilot data and similarly, these findings might be the basis of larger population controlled studies (Reichel et al. 1999; Spaide 2004). An example of this is the TTT pilot study of Reichel et al. (1999) which was based upon 16 eyes of 15 subjects and no control group. They demonstrated variability in results with respect to visual acuity, in so far as three eyes improved, four eyes showed a decline and nine eyes remained stable. One consistent finding was a decreased exudation on FFA in most eyes. Based upon this pilot study, treatment with TTT was “justified” and is now performed worldwide; the findings of Reichel et al.’ (1999)

study design has formed the basis for larger clinical trials which have just been started.

The experiments in this thesis have provided more knowledge about how retinal function is affected in ARM. The studies suggest that the cone- and rod-mediated mfERG could be a useful part of clinical routine examinations in ARM. Recently Jackson et al. (2004) investigated the full-field rod ERG in early and late ARM subjects. They could not find a significant effect in ARM compared to a young and old control group which was not surprising given the localized loss of rods which is not captured by a full-field ERG. They did not exclude the fact that the rod-mediated mfERG might be of value but stated “that it remains to be determined whether this technique can be reliably and validly implemented in studying ARM pathogenesis”. The results reported here show that the rod-mediated mfERG can be reliably used in studying ARM pathogenesis. However, further research is required before it could be used as a routine diagnostic tool. It is a demanding and time consuming procedure and different, more time effective approaches to isolate rods might be possible. For example, long wavelength backgrounds which suppress cone activity might be used additionally to low luminance, short wavelength stimuli for isolating rod responses.

7.2 Future directions

Monitoring retinal function with objective tests and correlating these to subjective measures should give more information on the level at which functional deficits first appear in early ARM. The goal is early detection before the full range of visual function is affected. The definition of early ARM is based upon funduscopy appearance and thus on gross anatomic features. These features may not always

reflect physiological changes with prognostic significance and other “signs of ARM” (e.g. hypoxia with post-receptoral and receptoral alterations) might only be captured with sensitive functional tests. Using a battery of visual function tests “with the advantage of depending directly on physiology as well as on anatomy” (Eisner et al. 1992) seems to be a better approach. A future approach might be investigating older subjects with normal fundi, based upon their risk factor history (e.g genetic predisposition, hypertension and smoking history) with a battery of subjective and objective function tests over a longer period of time. These tests should include function tests measuring rod- and cone-mediated adaptational dynamics such as dark adaptation and glare recovery which were not performed in this thesis experiments but might have additionally contributed to a better understanding of the pathomechanism in ARM.

New mfERG paradigms to study the effects on the later waveform components of ARM might be applied by using either inserted dark frames between consecutive frames alone and/or additional global flashes covering the entire stimulus array in ARM. While many protocols are still experimental and used to describe retinal properties (Wu and Sutter 1995; Sutter and Bearse 1998; Sutter et al. 1999; Bearse et al. 2000) some have been successfully applied in glaucoma (Fortune et al. 2002) and diabetes (Kurtenbach et al. 2000; Shimada et al. 2001; Onozu and Yamamoto 2003; Bearse et al. 2004a; Bearse et al. 2004b). Coloured mfERG arrays have been also introduced to isolate L- and M-cone contributions (Albrecht et al. 2002). Although these new mfERG paradigms have not yet become part of clinical routine, promising results suggest that they might become diagnostic and predictive tools in the future.

The treatment of early and late ARM remains a challenge and objective measures of retinal function are needed to document their effects. There is still insufficient knowledge about how dietary supplements, laser treatments, anti-angiogenic drugs, steroids or combinations of any of these therapies affect retinal function. Treatment dosage, number and effectiveness might be better estimated from functional findings. This might not only save costs but might also help in better understanding the condition and specific effects of the treatment.

Future treatments targeting the rods with for example high dosages of Vitamin A, or photoreceptor transplantation in early ARM remain hypothetical. However, the philosophy of Curcio et al. (2000) “Spare the rods, Save the cones...” might become an important approach in treating ARM. There is evidence of rod-cone interaction and protective effects of transplanted rods on host cones in animal experiments (Mohand-Said et al. 2001). Some classes of pharmaceuticals have been hypothesized to postpone or block rod death (LaVail et al. 1992; Liu et al. 1999) and might provide future treatment options. In future it might be necessary to have an accurate measure of rod-mediated function if new treatments emerge.

Another approach in the treatment of ARM might be supplemental oxygen. This approach is not new (Riva Sanseverino et al. 1990; Teichmann 1997) but large clinical trials have never been initiated to show its possible beneficial effect. Improvement of diabetic cystoid oedema measured with the OCT has been demonstrated after oxygen therapy in a recent study (Nguyen et al. 2004). It would be interesting to see if function improves during oxygen therapy measured with the rod- and cone-mediated mfERG in ARM.

In future, similar to a visual field testing, the mfERG could become an indispensable tool in clinical practise, not only reserved for research or specialized centres. It still needs to be determined how useful the mfERG will be in day-to-day practice where there are less time-consuming and sometimes stressful “performing” procedures for the patient. Recording and interpretation should be left to trained medical personnel, which might require employment of additional staff, but ultimately, the aim would be to simplify the mfERG to the point of a yes/no diagnostic test.

7.3 Conclusion

This thesis has introduced a new testing protocol for eliciting a rod-mediated mfERG and demonstrated delayed rod-mediated responses in early ARM compared to a healthy age-matched group. Based upon the previous literature and the experimental results of this thesis, a new hypoxia post-receptoral model is proposed.

The model suggests that in early ARM the cone- and rod-mediated post-receptoral pathways are primarily affected before the photoreceptors. Post-receptoral sites are more susceptible to ischemia and damage at these sites is thought to reflect synaptic transmission alterations between photoreceptors and bipolar cells. This transmission alteration is supported by delayed rod-mediated mfERG latencies which are mainly a bipolar cell response. A selective impairment of the S-cone post-receptoral pathways has been demonstrated in other ischemic diseases and in aging. Reduced HC-, LC- and low luminance visual acuity and impaired contrast sensitivity probably result from alteration of the horizontal cells mediating the lateral inhibitory mechanism also located post-receptorally.

Although retinal function is stabilized after multiple PDTs, it is hypothesized that choroidal hypoperfusion and hypoxia caused by repeated PDTs induce functional alteration of bipolar cells. This may be reflected in transiently decreased mfERG responses and a misshapened descending part of the cone-mediated mfERG positive waveform.

In accordance with funduscopy progression, the thesis findings show that progression of functional impairment is slow in early ARM. However, the hypoxia post-receptor ARM model suggests that further investigations based upon detecting and monitoring post-receptor dysfunction in ARM and in the treatment of ARM should be initiated.

MASTER REFERENCE LIST

- Abadi R, Pantazidou M (1996) Low contrast letter acuity in age-related maculopathy. *Ophthalmic Physiol Opt* 16:455-459
- Abdel-Meguid A, Lappas A, Hartmann K, Auer F, Schrage N, Thumann G, Kirchof B (2003) One year follow up of macular translocation with 360 degree retinotomy in patients with age-related macular degeneration. *Br J Ophthalmol* 87:615-621
- Abramson D, Abramson H (2002) High-dose supplements for age-related macular degeneration: did you leave out Centrum? *Arch Ophthalmol* 120:1602
- Adams A, Rodic R (1982) Use of desaturated and saturated version of the D-15 test in glaucoma and glaucoma-suspect patients. In Verriest, G (ed.), *Colour Vision Deficiencies VI, Doc Ophthalmol. Proc. The Hague: Dr. W. Junk* 33:419-424
- Adler J, Martin K (1983) Lysosomal enzymes in the interphotoreceptor matrix: acid protease. *Curr Eye Res* 2:359-365
- Age-Related Eye Disease Study Research Group (2001a) The age-related eye disease study system for classifying age-related macular degeneration from stereoscopic color fundus photographs: the Age-Related Eye Disease Study Report Number 6. *Am J Ophthalmol* 132:668-681
- Age-Related Eye Disease Study Research Group (2001b) The age-related eye disease study system for classifying cataracts from photographs: AREDS report number 4. *Am J Ophthalmol* 131:167-175
- Age-Related Eye Disease Study Research Group (2001c) A randomized, placebo-controlled, clinical trial of high dose supplementation with vitamin C and E, beta carotene, and zinc for age-related macular degeneration and vision loss. AREDS report no.8. *Arch Ophthalmol* 119:1417-1436
- Ahnelt P, Kolb H (1994) Horizontal cells and cone photoreceptors in human retina: a Golgi-electron microscopic study of spectral connectivity. *J Comp Neurol* 343:406-427
- Aiba T, Alpern M, Maaseidvaag F (1967) The electroretinogram evoked by excitation of human foveal cones. *J Phys* 189:43-62

- Aiello L, Northrup J, Keyt B, Takagi H, Iwamoto M (1995) Hypoxic regulation of vascular endothelial growth factor in retinal cells. *Arch Ophthalmol* 113:1538-1544
- Albrecht J, Jaegle H, Hood D, Sharpe L (2002) The multifocal electroretinogram (mfERG) and cone isolating stimuli: variation in L- and M-cone driven signals across the retina. *J Vision* 2:543-558
- Alge C, Priglinger S, Neubauer A, Kampik A, Zillig M, Bloemendal H, Welge-Lussen U (2002) Retinal pigment epithelium is protected against apoptosis by alphaB-crystallin. *Invest Ophthalmol Vis Sci* 43:3575-3582
- Algvere P (1997) Clinical possibilities in retinal pigment epithelium transplantations (editorial). *Acta Ophthalmol Scand* 75:1
- Algvere P, Berglin L, Gouras P, Sheng Y (1994) Transplantation of fetal retinal pigment epithelium in age-related macular degeneration with subfoveal neovascularisation. *Graefe's Arch Clin Exp Ophthalmol* 232:707-716
- Algvere P, Libert C, Lindgaerde G, Seregard S (2003) Transpupillary thermotherapy of predominantly occult choroidal neovascularisation in age-related macular degeneration with 12 months follow-up. *Acta Ophthalmol* 81:110-117
- Allikmets R, Shroyer N, Seddon J, Levis R, Bernstein P, Peiffer A, Zabriskie N, Li Y, Hutchinson A, Dean M, Lupski J, Leppert M (1997) Mutation of the Stargardt disease gene (ABCR) in age-related macular degeneration. *Science* 277:1805-1807
- Ames A, Li Y-Y, Heher E, Kimble C (1992) Energy metabolism of rabbit retina as related to function: high cost of NA⁺ transport. *J Neurosci* 12:840-853
- Anderson D, Mullins R, Hageman G, Johnson L (2002) A role for local inflammation in the formation of drusen in the aging eye. *Am J Ophthalmol* 134:411-431
- Anzai K, Mori K, Murayama K, Yoneya S (1997) Normal values and their variation with age in multifocal electroretinogram. 1997 Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO) 38:Abstract 4116
- Apostolopoulos M, Koutsandrea C, Moschos M, Alonistiotis D, Papaspyrou A, Mallias J, Kyriaki T, Theodossiadis P, Theodossiadis G (2002) Evaluation of successful macular hole surgery by optical coherence tomography and multifocal electroretinography. *Am J Ophthalmol* 134:667-674

- Applegate R, Adams A, Cavender J, Zisman F (1987) Early color vision changes in age-related maculopathy. *Appl Opt* 26:1458-1462
- Arai M, de Faria J, Hirose T (1999) Effects of stimulus blocking, light scattering, and distortion on multifocal electroretinogram. *Jpn J Ophthalmol* 43:481-489
- Arai M, Nobuhisa N, Sawada A, Hayashida T (1998) Multifocal electroretinogram indicates visual field loss in acute zonal occult outer retinopathy. *Am J Ophthalmol* 126:466-469
- Arden G, Banks J (1966) Foveal electroretinogram as a clinical test. *Br J Ophthalmol* 50:740
- Arden G, Wolf J, Tsang Y (1998) Does dark adaptation exacerbate diabetic retinopathy? Evidence and a linking hypothesis. *Vision Res* 38:1723-1729
- Attebo K, Mitchell P, Smith W (1996) Visual acuity and the causes of visual loss in Australia. The Blue Mountains Eye Study. *Ophthalmol* 103:357-364
- Bailey I, Lovie J (1976) New design principles for visual acuity letter charts. *Am J Optom Physiol Opt* 53:740-745
- Bailey I, Lovie J (1980) The design and use of a new-near vision chart. *Am J Optom Physiol Opt* 57:378-387
- Bartlett H, Eperjesi F (2003) Age-related macular degeneration and nutritional supplementation: a review of randomised controlled trials. 23:383-399
- Baseler H, Schneck M, Sutter E (1996) Contribution of different receptor populations to multifocal ERGs and VEPs. 1996 Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO):1061, Abstract 4876
- Bazan N (1989) The metabolism of omega-3 polyunsaturated fatty acids in the eye: the possible role of docosahexaenoic acid and docosanoids in retinal physiology and ocular pathology. *Prog Clin Biol Res* 312:95-112
- Bearse M, Sutter E (1996) Imaging localized retinal dysfunction with the multifocal electroretinogram. *J Opt Soc Am A* 13:634-640
- Bearse M, Sutter E (1998) Contrast dependence of multifocal ERG components. In *Vision Science and its Application*. OSA Tech Dig Ser, Optical Society of America, Washington, DC 1:24-27
- Bearse M, Sutter E, Lerner L (1994) Imaging retinal damage with the multi-input electroretinogram. In *Vision science and its application*, OSA Tech Dig Ser, Optical Society of America, Washington, DC:358-361

- Bearse M, Sutter E, Sim D, Stamper R (1996) Glaucomatous dysfunction revealed in higher order components of the electroretinogram. In Vision science and its application, OSA Tech Dig Ser, Optical Society of America, Washington, DC 1:104-107
- Bearse M, Sutter E, Smith D, Rose S (1995a) Early detection of macular dysfunction in the topography of the electroretinogram. In Vision Science and its Applications. OSA Tech Dig Ser, Optical Society of America, Washington, DC 2:318-321
- Bearse M, Sutter E, Smith D, Stamper R (1995b) Ganglion cell components of the human multi-focal ERG are abnormal in optic nerve atrophy and glaucoma. Invest Ophthalmol Vis Sci 36:445
- Bearse MJ, Han Y, Schneck M, Adams A (2004a) Retinal function in normal and diabetic eyes mapped with slow flash multifocal electroretinogram. Invest Ophthalmol Vis Sci 45:296-304
- Bearse MJ, Han Y, Schneck M, Barez S, Jacobson C, Adams A (2004b) Local multifocal oscillatory potential abnormalities in diabetes and early diabetic retinopathy. Invest Ophthalmol Vis Sci 45:3259-3265
- Bearse MJ, Shimada Y, Sutter E (2000) Distribution of oscillatory components in the central retina. Doc Ophthalmol 100:185-205
- Bearse MJ, Sutter E, Palmowski A (1997) Luminance-dependent enhancement of ganglion cell contributions to the human multifocal ERG. 1997 Annual Meeting of the Association for Research in Vision and Ophthalmology 38:959
- Beatty S, Boulton M, Henson D, Koh H, Murray I (1999) Macular pigment and age-related macular degeneration. Br J Ophthalmol 83:867-877
- Beatty S, Koh H, Carden D, Murray I (2000a) Macular pigment optical density measurement: a novel compact instrument. Ophthalmic Physiol Opt 20:105-111
- Beatty S, Koh H, Henson D, Boulton M (2000b) The role of oxidative stress in the pathogenesis of age-related macular degeneration. Surv Ophthalmol 45:115-132
- Berendschot T, Goldbohm R, Klopping W, van de Kraats J, van Norel J, van Norren D (2000) Influence of lutein supplementation on macular pigment, assessed with two objective techniques. Invest Ophthalmol Vis Sci 41:3322-3326

- Besch D, Kurtenbach A, Apfelstedt-Sylla E, Sadowski B, Dennig D, Asenbauer C, Zrenner E, Schiefer U (2002) Visual field constriction and electrophysiological changes associated with vigabatrin. *Doc Ophthalmol* 104:151-170
- Bhutto I, Hasegawa T, Kim S, McLeod D, Merges C, Tong P, Luty G (2004) Expression of pigmentepithelium-derived factor (PEDF) in human aged choroid and age-related macular degeneration. 2004 Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO):Abstract 1786
- Biersdorf W, Diller D (1969) Local electroretinogram in macular degeneration. *Am J Ophthalmol* 68:269-303
- Binder S, Stolba U, Krebs I, Kellner L, Jahn C, Feichtinger H, Povelka M, Frohner U, Kruger A, Hilgers R-D, Krugluger W (2002) Transplantation of autologous retinal pigment epithelium in eyes with foveal neovascularisation resulting from age-related macular degeneration: a pilot study. *Am J Ophthalmol* 133:215-225
- Bird A (1991) Pathogenesis of retinal pigment epithelial detachment in the elderly; the relevance of Bruch's membrane change. *Eye* 5:1-12
- Bird A (2001) The aging macula. *Eye* 15:369-370
- Bird A (2003) Age-related macular disease: an ongoing challenge. *Clin Exp Ophthalmol* 31:461-463
- Bird A, Bressler N, Bressler S, Chisholm I, Coscas G, Davis M, de Jong P, Klein B, Klein R (1995) An international classification and grading system for age-related maculopathy and age-related macular degeneration. The International ARM Epidemiological Study Group. *Surv Ophthalmol* 39:367-374
- Bird A, Marshall J (1986) Retinal pigment epithelial detachments in the elderly. *Trans Am Ophth Soc* 105:674-682
- Blaauweegers H, Holtkamp G, Rutten H, Witmer AN, Koolwijk P, Patanen A, Alitalo K, Kroon M, Kijlstra A, van Hinsberg V, Schlingemann R (1999) Polarized vascular endothelial growth factor secretion by human retinal pigment epithelium and localisation of vascular endothelial growth factor receptors on the inner choriocapillaris. Evidence for a paracrine relation. *Am J Pathol* 155:412-428

- Blanks J, Torigoe Y, Hinton D, Blanks R (1991) Retinal degeneration in the macula of patients with Alzheimer's disease. *Ann N Y Acad Sci* 640:44-46
- Block F, Schwarz M (1998) The b-wave of the electroretinogram as an index of retinal ischemia. *Gen Pharmacol* 30:281-287
- Bloome M (1980) Fluorescein angiography: Risks. *Vision Res* 20:1083-1097
- Blumenkranz M, Bressler N, Bressler S, Treatment of Age-Related Macular Degeneration With Photodynamic Therapy (TAP) study group (2002) Verteporfin therapy for subfoveal choroidal neovascularization in age-related macular degeneration: three-year results of an open-label extension of 2 randomized clinical trials--TAP Report no. 5. *Arch Ophthalmol* 120:1307-1314
- Blumenthal R (2000) Statins: effective antiatherosclerotic therapy. *Am Heart J* 139:577-583
- Bock M, Gerth C, Lorenz B (2000) Impact of notch filter use on waveforms of first- and second-order-kernel responses from multifocal ERGs. *Doc Ophthalmol* 101:195-210
- Borger P, van Leeuwen R, Hulsman C, Wolfs R, van der Kuip D, Hofman A, de Jong P (2003) Is there a direct association between age-related eye disease and mortality? The Rotterdam Study. *Ophthalmology* 110:1292-1296
- Boulton M (1991) Aging of the retinal pigment epithelium. In: Osborne NN, Chader GJ, (eds.), *Progress in retinal research*. Oxford: Pergamon Press 11:125-152
- Boulton M, Dayhaw-Barker P (2001) The role of the retinal pigment epithelium: topographical variations and ageing changes. *Eye* 15:384-389
- Boulton M, Donstov A, Jarvis-Evans J, Ostovsky M, Svistunenko D (1993) Lipofuscin is a photoinducible free radical generator. *Photochem Photobiol B* 19:201-204
- Bowman K, Cameron K (1984) A quantitative assessment of colour discrimination in normal and senile macular degeneration using some colour confusion tests. In Verriest, G (ed.), *Colour Vision Deficiencies VII*, *Doc Ophthalmol. Proc. The Hague: Dr. W. Junk* 39:363-370
- Boycott B, Wässle H (1991) Morphological classification of bipolar cells of the primate retina. *Eur J Neurosci.* 11:1069-1088
- Boynton R (1952) Stray light and the human electroretinogram. *J Opt Soc Am A* 43:442-444

- Brenton R, Phelps C, Rojas P, Woolson R (1986) Interocular differences of the visual field in normal subjects. *Invest Ophthalmol Vis Sci* 27:799-805
- Bressler N, Bressler S, Congdon N, Ferris Fr, Friedman D, Klein R, Lindblad A, Milton R, Seddon J, Age-Related Eye Disease Study Research Group (2003) Potential public health impact of age-related eye disease study results: AREDS report no. 11. *Arch Ophthalmol* 121:1634-1636
- Bressler N, Bressler S, Gragoudas E (1987) Clinical characteristics of choroidal neovascular membranes. *Arch Ophthalmol* 105:209-213
- Bressler N, Munoz B, Maguire M, Vitale S, Schein O, Taylor HR, SK W (1995) Five-year incidence and disappearance of drusen and retinal pigmentepithelial abnormalities. Waterman Study. *Arch Ophthalmol* 113:301-308
- Bressler N, Silva J, Bressler S, Fine S, Green W (1994) Clinicopathologic correlation of drusen and retinal pigment epithelial abnormalities in age-related macular degeneration. *Retina* 14:130-142
- Bressler N, Treatment of Age-Related Macular Degeneration With Photodynamic Therapy (TAP) Study Group (2001) Photodynamic therapy of subfoveal choroidal neovascularization in age-related macular degeneration with verteporfin: two-year results of 2 randomized clinical trials-tap report 2. *Arch Ophthalmol* 119:198-207
- Bressler S, Bressler N, Fine S (1982) Natural course of choroidal neovascularisation membranes within the foveal avascular zone in senile macular degeneration. *Am J Ophthalmol*:157-163
- Bressler S, Pieramic D, Bressler N (2004) Natural history of minimally classic subfoveal choroidal neovascular lesions in the treatment of age-related macular degeneration with photodynamic therapy--TAP report No.6. *Arch Ophthalmol* 122:325-329
- Brown B, Adams A, Coletta N, Haegerstrom-Portnoy G (1986a) Dark adaptation in age-related maculopathy. *Ophthalmic Physiol Opt* 6:81-84
- Brown B, Garner L (1983) Effects of luminance on contrast sensitivity in senile macular degeneration. *Am J Optom Physiol Opt* 60:788-793
- Brown B, Kitchin J (1983) Dark adaptation and the acuity/luminance response in senile macular degeneration (SMD). *Am J Optom Physiol Opt* 60:645-650

- Brown B, Lovie-Kitchin J (1987a) Contrast sensitivity in central and paracentral retina in age-related maculopathy. *Clin Exp Opt* 70:145-148
- Brown B, Lovie-Kitchin J (1987b) Temporal function in age related maculopathy. *Clin Exp Opt* 70:112-116
- Brown B, Tobin C, Roche N, Wolanowski A (1986b) Cone adaptation in age-related maculopathy. *Am J Optom Physiol Opt* 63:450-454
- Brown B, Yap M (1995) Contrast and luminance as parameters defining the output of the VERIS topographical ERG. *Ophthal Physiol Opt* 16:42-48
- Bui BV, Vingrys A, Kalloniatis M (2003) Correlating retinal function and amino acid immunocytochemistry following post-mortem ischemia. *Exp Eye Res* 77:125-136
- Bumsted K, Hendrickson A (1999) Distribution and development of short-wavelength cones differ between macaca monkey and human fovea. *J Comp Neurol* 403:502-516
- Bunse A, Bock M, Lorenz B, Gabel V (2001) Photodynamic therapy in age-related macular degeneration: functional results. 2001 Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO) 42:Abstract 2379
- Burns S, Elsner A, Weiter J, Kreitz M (1991) Cone photopigment density in aging and AMD. In: *Noninvasive Assessment of the Visual System*. OSA Technical Digest Series Optical Society of America, Washington, DC 1:194-197
- Cai J, Nelson K, Wu M, Sternberg P, Jones D (2000) Oxidative damage and protection of the RPE. *Prog Retin Eye Res* 19:205-221
- Campbell C, Ritter M (1969) Clinical adaptation studies of the human retina. In Straatsma, BR, Hall, MO, Allen, RA and Crescitelli, F (eds.), *The Retina. Morphology, Function and Clinical Characteristics*. University of California Press, Berkley:513-544
- Campochiaro P (1998) Growth factors in the retinal pigment epithelium and retina. In: Marmor M, Wolfensberger, T, (eds.) *The retinal pigment epithelium*. Oxford: Oxford University Press.:459-477.
- Campochiaro P (2000) Retinal and choroidal neovascularisation. *J Cell Physiol* 181:301-310

- Carter-Dawson L, Kuwabara T, O'Brien P, Bieri J (1979) Structural and biochemical changes in vitamin A deficient rats retinas. *Invest Ophthalmol Vis Sci* 18:437-446
- Casten R, Rovner B, Tasman W (2004) Age-related macular degeneration and depression: a review of recent research. *Curr Eye Res* 15:181-183
- Challa J, Gillies M, Penfold PL, Gyory J, Hunyor A, Billson F (1998) Exudative macular degeneration and intravitreal triamcinolone: 18 months follow up. *Aust NZ J Ophthalmol.* 26:277-281
- Chalupa L, Guenthan E (2004) Development of ON and OFF retinal pathways and retinogeniculate projections. *Prog Ret Eye Res* 23:31-51
- Chan C, Brown B (1999) Multifocal ERG changes in glaucoma. *Ophthalm Physiol Opt* 19:306-316
- Chan H, Brown B (1998) Investigation of retinitis pigmentosa using the multifocal electroretinogram. *Ophthalm Physiol Opt* 18:335-350
- Chan H, Sui A, Yap M, Brown B (2002) The effect of light scattering on the multifocal electroretinogram. *Ophthalm Physiol Opt* 22:482-490
- Chan HHL, Brown B (2000) Pilot study of the multifocal electroretinogram in ocular hypertension. *Br J Ophthalmol* 84:1147-1153
- Chappelow A, Marmor M (2000) Multifocal electroretinogram abnormalities persist following resolution of central serous chorioretinopathy. *Arch Ophthalmol* 118:1211-1215
- Chen C, Wu L, Wu D, Huang S, Wen F, Luo G, Long S (2004) The local cone and rod system function in early age-related macular degeneration. *Doc Ophthalmol* 109:1-8
- Chen H, Wu L, Pan S, Wu D (1993) An immunologic study on age-related macular degeneration. *Yan Ke Xue Bao* 9:113-120
- Chen J, Fitzke F, Pauleikhoff D, Bird A (1992) Functional loss in age-related Bruch's membrane change with choroidal perfusion defect. *Invest Ophthalmol Vis Sci* 33:334-340
- Cheng A, Vingrys A (1992) Visual losses in early age-related maculopathy. *Optom Vis Sci* 70:89-96
- Cho E, Hung S, Willett W, Spiegelman D, Rimm E, Seddon J, Colditz G, Hankinson S (2001) Prospective study of dietary fat and the risk of age related macular degeneration. *Am J Clin Nutr* 73:209-218

- Cho E, Seddon J, Rosner B, Willett W, Hankinson S (2004) Prospective study of intake of fruits, vegetables, vitamins, and carotenoids and risk of age-related macular degeneration. *Arch Ophthalmol* 122:883-892
- Choroidal Neovascularization Prevention Trial Research Group (2003) Laser treatment in fellow eyes with large drusen: updated findings from a pilot randomized clinical trial. *Ophthalmology* 110:971-978
- Cimbalas A, Cerniauskiene L, Paunksnis A, Tamosiunas A, Luksiene D, Saferis V (2004) Association of age-related maculopathy with ischemic heart disease and its risk factors in middle-aged population of Kaunas city. [Article in Lithuanian]. *Medicina (Kaunas)*. 40:671-676
- Ciulla T, Harris A, Chung H (1999) Color doppler imaging discloses reduced ocular blood flow velocities in non-exudative age-related macular degeneration. *Am J Ophthalmol* 128:75-80
- Ciulla T, Harris A, Kagemann L, Danis R, Maturi R, McNulty L, Pratt L, Xiao M, Criswell M, Weinberger D (2001a) Transpupillary thermotherapy for subfoveal occult choroidal neovascularization: effect on ocular perfusion. *Invest. Ophthalmol Vis Sci* 42:3337-3340
- Ciulla T, Harris A, Martin B (2001b) Ocular perfusion and age-related macular degeneration. *Acta Ophthalmol Scand* 79:108-115
- Ciulla TA (2003) Recent advances in the treatment of exudative age-related macular degeneration, including transpupillary thermotherapy. *Acta Ophthalmol Scand* 81:103-104
- Clark A (1997) AL-3789: a novel ophthalmologic angiostatic steroid. *Exp Opin Invest Drugs* 124:521-529
- Coile D, Baker H (1992) Foveal dark adaptation, photopigment regeneration, and aging. *Vis Neurosci* 8:27-39
- Coleman K, Fitzgerald D, Eustace P, Bouchier-Hayes D (1990) Electroretinography, retinal ischemia and carotid artery disease. *Eur J Vasc Surg* 4:13-18
- Collins M (1986) Pre-age-related maculopathy and the desaturated D-15 colour vision test. *Clin Exp Opt* 69:223-227
- Collins M, Brown B (1989a) Glare recovery and age-related maculopathy. *Clin Vis Sci* 4:145-153
- Collins M, Brown B (1989b) Glare recovery and its relation to other clinical findings in age related maculopathy. *Clin Vis Sci* 4:155-163

- Committee on Vision AoBaSS, National Research Council. (1981) Procedures for testing color Vision: report of working group 41. Washington, D.C. National Academy Press:57-60
- Connolly B, Regillo C, Eagle R, Jr., Shields C, Shields J, Moran H (2003) The histopathologic effects of transpupillary thermotherapy in human eyes. *Ophthalmology* 110:415-420
- Costa R, Farah J, Cardillo J, Calucci D, Williams G (2003) Immediate indocyanine green angiography and optical coherence angiography evaluation after photodynamic therapy for subfoveal choroidal neovascularisation. *Retina* 23:159-165
- Coupland S (1991) Electrodes for clinical electrophysiology testing. In: Heckenlively JR and Arden GB (eds.), *Principle and Practice of Clinical Electrophysiology of Vision*. St Louis: Mosby:177-182
- Crabb J, Miyagi M, Gu X, Shadrach K, West K, Sakaguchi H, Kamei M, Hasan A, Yan L, Rayborn M, Salomon R, Hollyfield J (2002) Drusen proteome analysis: an approach to the aetiology of age-related macular degeneration. *Proc Natl Acad Sci USA* 99:14682-14687
- Crawford B (1972) The Stiles Crawford effects and their significance in vision. In: Jameson D, Hurvich L, (eds.), *Handbook of Sensory Physiology. Visual Psychophysics*. New York: Springer-Verlag VII/4:470-483
- Cringle S, Yu D-Y, Yu P, Su E-N (2002) Intraretinal oxygen consumption in the rat in vivo. *Invest Ophthalmol Vis Sci*. 43:1922-1927
- Cruickshanks K, Hamman R, Klein R, Nondahl D, Shetterly S (1997) The prevalence of age-related maculopathy by geographic region and ethnicity. The Colorado-Wisconsin Study of Age-Related Maculopathy. *Arch Ophthalmol* 115:242-250
- Cruickshanks K, Klein R, Klein B (1993) Sunlight and age-related macular degeneration. The Beaver Dam Eye Study. *Arch Ophthalmol* 111:514-518
- Cruickshanks K, Klein R, Klein B, Nondahl D (2001) Sunlight and the 5-year incidence of early age-related maculopathy: the Beaver Dam Eye Study. *Arch Ophthalmol* 119:246-250
- Csaky K (2003) Anti-vascular endothelium growth factor therapy for neovascular age-related macular degeneration. Promises and pitfalls. *Ophthalmology* 110:879-880

- Curcio C (2001) Photoreceptor topography in aging and age-related maculopathy. *Eye* 15:376-383
- Curcio C, Medeiros N, Millican L (1996) Photoreceptor loss in age-related macular degeneration. *Invest Ophthalmol Vis Sci* 37:1236-1249
- Curcio C, Millican C (1999) Basal linear deposit and large drusen are specific for early age-related maculopathy. *Arch Ophthalmol* 117:329-339
- Curcio C, Millican L, Allen K, Kalina R (1993) Aging of the human photoreceptor mosaic: evidence for selective vulnerability of rods in central retina. *Invest Ophthalmol Vis Sci* 34:3278-3296
- Curcio C, Millican L, Bailey T, Kruth H (2001) Accumulation of cholesterol with age in human Bruch's membrane. *Invest Ophthalmol Vis Sci* 42:265-274
- Curcio C, Owsley C, Jackson G (2000) Spare the rods, save the cones in aging and age-related maculopathy. *Invest Ophthalmol Vis Sci* 41:2015-2018
- Curcio C, Sloan K, Kalina R, Hendrickson A (1990) Human photoreceptor topography. *J Comp Neurol* 292:497-523
- Dacey D, Lee B (1994) The 'blue-on' opponent pathway in primate retina originates from a distinct bistratified ganglion cell type. *Nature* 367:731-735
- D'Amico D, Goldberg M, Hudson H, Jerdan J, Krueger D, Luna S, Robertson S, Russell S, Singerman L, Slakter J, Yannuzzi L, Ziliox P, Anecortave Acetate Clinical Study Group (2003) Anecortave acetate as monotherapy for the treatment of subfoveal lesions in patients with exudative age-related macular degeneration (AMD): interim (month 6) analysis of clinical safety and efficacy. *Retina* 23:14-23
- Danis R, Ciulla T, Pratt L, Anliker W (2000) Intravitreal triamcinolone acetonide in exudative age-related macular degeneration. *Retina* 20:244-250
- Dargel H (1992) Lipid peroxidation-a common pathogenetic mechanism? *Exp Toxicol Pathol* 44:169-181
- Darzins P, Mitchell P, Heller R (1997) Sun exposure and age-related macular degeneration. An Australian case-control study. *Ophthalmology* 104:770-776
- Dawson D, Volpert O, Gillis P, Crawford S, Xu H, Benedict W, Bouck N (1999) Pigment epithelium-derived factor: a potent inhibitor of angiogenesis. *Science* 284:1034-1037
- De Jong P, Bergen A, Klaver C, van Duijn C, Assink J (2001) Age-related maculopathy: its genetic basis. *Eye* 15:396-400

- De La Paz M, Guy V, Abu-Donia S, Heinis R, Bracken B, Vance J, Gilbert J, Gass J, Haines J, Pericak-Vance M (1999) Analysis of the Stargardt disease gene (ABCR) in age-related macular degeneration. *Ophthalmology* 106:1531-1536
- De Monasterio F, Gouras P (1975) Functional properties of ganglion cells of the rhesus monkey retina. *J Physiol Lon* 251:167-195
- de Waard P, Ij J, van de Berg T, de Jong P (1992) Intraocular light scattering in age-related cataracts. *Invest Ophthalmol Vis Sci* 33:618-625
- Del Priore L, Kuo Y-H, Tezel T (2002) Age-related changes in human RPE cell density and apoptosis proportion in situ. *Invest Ophthalmol Vis Sci* 43:3311-3318
- Delori F, Dorey C, Staurengi G, Arend O, Goger D, Weiter J (1995) In vivo fluorescence of the ocular fundus exhibits retinal pigmentepithelium lipofuscin characteristics. *Invest Ophthalmol Vis Sci* 36:718-729
- Demelle M (1978) Retinal sensitized photodynamic damage to liposomes. *Photochem Photobiol* 28:357-360
- Dentchev T, Milam AH, Lee VM, Trojanowski JQ, JL D (2003) Amyloid-beta is found in drusen from some age-related macular degeneration retinas, but not in drusen from normal retinas. *Mol Vis* 9:184-190
- DeVries S (2000) Bipolar cells use kainate and AMPA receptors to filter visual information into separate channels. *Neuron* 28:847-856
- Dolan F, Parks S, Keating D, GN. D, Evans A (2003) Multifocal electroretinographic features of central retinal vein occlusion. *Invest Ophthalmol Vis Sci* 2003:4954-4959
- Donatien P, Jeffery G (2002) Correlation between rod photoreceptor numbers and levels of ocular pigmentation. *Invest Ophthalmol Vis Sci* 43:1198-1203
- Dorey C, Wu G, Ebenstein D, Garsd A, Weiter J (1989) Relationship to lipofuscin accumulation and age-related macular degeneration. *Invest Ophthalmol Vis Sci* 30:1691-1699
- Dowling J, Ehinger B (1975) Synaptic organisation of the amine-containing interplexiform cells of the goldfish and Cebus monkey retina. *Science* 188:270-273
- Dowling J, Wald G (1958) Vitamin A deficiency and night blindness. *Proc Natl Acad Sci USA* 44:648-661

- Doyne R (1899) A peculiar condition of choroiditis occurring in several members of the same family. *Trans Ophthalmol Soc UK* 19:17
- Doyne R (1910) A note on family choroiditis. *Trans Am Ophth Soc UK* 30:93-95
- Drexler W, Morgner U, Ghanta R, Kartner F, Schuman J, Fujimoto J (2001) Ultrahigh-resolution ophthalmic optical coherence tomography. *Nat Med* 7:502-507
- Dubois-Poulsen A, Lanthony P (1973) Le Farnsworth-15 desature'. *Bull Soc Ophthalmol Fr* 73:861-866
- Dumonde D, Kasp-Grochowska E, Graham E, Dsanders M, Faure J-P, de Kozak Y, van Tuyen V (1982) Anti-retinal autoimmunity and circulating immune complexes in patients with retinal vasculitis. *Lancet* 2:787-790
- Dunaief J, Dentehev T, Ying G-S, Milam A (2002) The role of apoptosis in age-related macular degeneration. *Arch Ophthalmol* 120:1435-1442.
- Duncan K, Bailey K, Kane J, Schwartz D (2002) Human retinal pigment epithelium express scavenger receptors BI and BII. *Biochem Biophys Res Commun* 292:1071-1022
- Duvall J, Tso M (1985) Cellular mechanisms of resolution of drusen after laser coagulation: an experimental study. *Arch Ophthalmol* 103:694-703
- Ebrey T, Koutalos Y (2000) Vertebrate photoreceptors. *Prog Retin Eye Res* 20:49-94
- Eckhard C, Eckhard U, Conrad H (1999) Macular rotation with and without counter rotation of the globe in patients with age-related macular degeneration. *Graefe's Arch Clin Exp Ophthalmol* 237:313-325
- Einbock W, Moessner A, Schnurrbusch U, Holz F, Wolf S, FAM-Study Group (2004) Different types of fundus autofluorescence in patients with age-related maculopathy correlated to risk of loss of visual acuity: prospective study. 2004 Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO):Abstract 2964
- Eisner A, Fleming S, Klein M, Mauldin W (1987a) Sensitivities in older eyes with good acuity: cross-sectional norms. *Invest Ophthalmol Vis Sci* 28:1824-1832
- Eisner A, Fleming S, Klein M, Mauldin W (1987b) Sensitivities in older eyes with good acuity: eyes whose fellow eye has exudative AMD. *Invest Ophthalmol Vis Sci* 28:1832-1837

- Eisner A, Klein M, Zilis J, Watkins M (1992) Visual function and the subsequent development of exudative age-related macular degeneration. *Invest Ophthalmol Vis Sci* 33:3091-3102
- Eisner A, Stoumbos V, Klein M, Fleming A (1991) Relation between fundus appearance and function. Eyes whose fellow eye has exudative age-related macular degeneration. *Invest Ophthalmol Vis Sci* 32:8-20
- Eldred G, Katz J (1988) Fluorophores of the human retinal pigment epithelium: separation and spectral characterization. *Exp Eye Res* 47:71-86
- Eldred G, Lasky M (1993) Retinal age pigment generated by self-assembling lysosomotropic detergents. *Nature* 361:724-726
- Elledge J, Blodi B, Ip M, Reichel E, Musch D, TTT4CNV Study (2004) Transpupillary thermotherapy of occult subfoveal choroidal neovascularisation membranes in patients with age-related macular degeneration (TTT4CNV): study design and patient baseline characteristics. 2004 Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO):Abstract 3060
- Elliott D, Whitaker D, Bonette L (1990) Differences in the legibility of letters at contrast threshold using the Pelli-Robson chart. *Ophthal Physiol Opt* 10:323-326
- Elsner A, Burns S (2002) Cone photopigment in older subjects: decreased optical density in early age-related macular degeneration. *J Opt Soc Am A* 19:215-222
- Elsner A, Burns S, Weiter J, Delori F (1995) Infrared imaging of sub-retinal structures in the human ocular fundus. *Vision Res* 36:191-205
- Elsner H, Schmidt-Erfurth U (2002) Analysis of fixation results after photodynamic therapy of subfoveal choroidal neovascularisation [in German]. *Ophthalmologie* 99:620-624
- Ergun E, Maar N, Radner W, Barbarzetto I, Schmidt-Erfurth U (2003) Scotoma size and reading speed in patients with subfoveal occult choroidal neovascularization in age-related macular degeneration. *Ophthalmology* 110:65-69
- Ermakov I, Ermakova M, Gellermann W, Bernstein P (2004) Macular pigment Raman detector for clinical applications. *J Biomed Opt* 9:139-148

- Esakowitz L, Kriss A, Shawkat F (1993) A comparison of flash electroretinograms recorded from Burian Allen, JET, C-glide, gold foil, DTL and skin electrodes. *Eye* 7:169-171
- Euler T, Wässle H (1995) Immunocytochemical identification of cone bipolar cells in the rat retina. *J Comp Neurol* 361:461-478
- Evans J (1995) Causes of blindness and partial sight in England and Wales 1990-1991. *Studies on medical and population subjects No.57. Studies on medical and population subjects, no 57.* Her Majesty's Stationary Office, London, UK.
- Evans J (2000) Risk factors for age-related macular degeneration. *Prog Ret Eye Res* 20:227-253
- Evans J, Wormald R (1996) Is the incidence of registrable age-related macular degeneration increasing? *Br J Ophthalmol* 80:9-14
- Eyetech Study G (2002) Preclinical and phase 1A clinical evaluation of an anti-VEGF pegylated aptamer (EYE001) for the treatment of exudative age-related macular degeneration. *Retina* 22:143-152
- Eyetech Study G (2003) Anti-vascular endothelial growth factor therapy for subfoveal choroidal neovascularization secondary to age-related macular degeneration: phase II study results. *Ophthalmology* 110:979-986
- Fain G, Ishida A, Callery S (1983) Mechanisms of synaptic transmission in the retina. *Vision Res* 23:1239-1249
- Falsini B, Fadda A, Iarossi G, Piccardi M, Canu D, Minnella A, Serrao S, Scullica L (2003) Retinal sensitivity to flicker modulation: reduced by early age-related maculopathy. *Invest Ophthalmol Vis Sci* 41:1498-1506
- Falsini B, Riva C, Logean E (2002) Flicker-evoked changes in human optic nerve blood flow: relationship with retinal neural activity. *Invest Ophthalmol Vis Sci* 43:2309-2316
- Famiglietti E, Kolb H (1976) Structural basis for ON-and OFF-center responses in retinal ganglion cells. *Science* 194:193-195
- Farkas T, Sylvester V, Archer D (1971) The ultrastructure of drusen. *Am J Ophthalmol* 71:1196-1205
- Feeney L (1978) Lipofuscin and melanin of human retinal pigment epithelium. Fluorescence, enzyme cytochemical and ultrastructural studies. *Invest Ophthalmol Vis Sci* 17:583-600

- Feeney-Burns L, Berman E, Rothman H (1980) Lipofuscin of human retinal pigment epithelium. *Am J Ophthalmol* 90:783-791
- Feeney-Burns L, Burns R, Gao C (1990) Age-related macular changes in human eyes over 90 years old. *Am J Ophthalmol* 109
- Feeney-Burns L, Hilderbrand E, Elridge S (1984) Aging human RPE: morphometric analysis of macular, equatorial and peripheral cells. *Invest Ophthalmol Vis Sci* 25:195-200
- Feigl B, Brown B, Lovie-Kitchin J, Swann P (2004a) Cone-and rod-mediated mfERG in early age-related maculopathy. *Eye advanced online publication*
- Feigl B, Brown B, Lovie-Kitchin J, Swann P (2004b) Cone-mediated multifocal electroretinogram in early age-related maculopathy and its relationships with subjective macular function tests. *Curr Eye Res* 29:327-336
- Feigl B, Brown B, Lovie-Kitchin J, Swann P (2004c) Monitoring retinal function in early age-related maculopathy: visual performance after one year. *Eye advanced online publication*
- Feigl B, Haas A (2001) Multifocal ERG in central areolar choroideal dystrophy. [in German]. *Ophthalmologie* 98:1074-1078
- Feigl B, Haas A, El-Shabrawi Y (2002) Multifocal ERG in multiple evanescent white dot syndrome. *Graefe's Arch Clin Exp Ophthalmol* 240:615-621
- Felix C, Hyde J, Sealy R (1979) Photoreactions of melanin. A new transient species and evidence for triplet state involvement. *Biochem Biophys Res Commun* 88:456
- Figuroa M, Schocket L, DuPont J, Metelitsina T, Grunwald J (2004) Effect of laser treatment for dry age related macular degeneration on foveolar choroidal hemodynamics. *Br J Ophthalmol* 88:792-795
- Flood V, Smith W, Wang J, Manzi F, Webb K, Mitchell P (2002) Dietary antioxidant intake and incidence of early age-related maculopathy. The Blue Mountains Eye Study. *Ophthalmology* 109:2272-2278
- Folkman J, Ingber D (1987) Angiostatic steroids. *Ann Surg* 206:374-382
- Fortune B, Bearnse MJ, Cioffi G, Johnson C (2002) Selective loss of an oscillatory component from temporal retinal multifocal ERG responses in glaucoma. *Invest Ophthalmol Vis Sci* 43:2638-2647

- Fortune B, Johnson C (2002) Decline of photopic multifocal electroretinogram responses with age is due to preretinal optical factors. *J Opt Soc Am A Opt Image Sci Vis* 9:173-183
- Fortune B, Johnson C, Cioffi G (2001) The topographic relationship between multifocal electroretinographic and behavioural perimetric measures of function in glaucoma. *Optom Vis Sci* 74:206-214
- Fortune B, Schneck M, Adams A (1999) Multifocal electroretinogram delays reveal local retinal dysfunction in early diabetic retinopathy. *Invest Ophthalmol Vis Sci* 40:2638-2651
- Frank R, Puklin J, Stock C, Canter L (2000) Race, iris color, and age-related macular degeneration. *Trans Am Ophthalmol Soc* 98:109-117
- Fredette M-J, Shekavat H, Lalonde G, Cinq-Mars B, Tardif Y, Malenfant, M., Hebert M (2003) Can the mfERG be used to follow-up AMD patients after PDT and predict the need for retreatment. 2003 Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO):Abstract 1831
- Fredette M-J, Shekavat H, Sasseville A, Grondin V, Lalonde G, Hebert M (2004) Multifocal ERG: a supplemental tool to follow patients treated with photodynamic therapy (PDT). 2004 Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO):Abstract 3174
- Freeman E, Munoz B, West S, Tielsch J, Schein O (2003) Is there an association between cataract surgery and age-related macular degeneration? Data from three population-based studies. *Am J Ophthalmol* 135:849-856
- Frennesson C, Nilsson U, Nilsson S (1995) Colour contrast sensitivity in patients with soft drusen, an early stage of ARM. *Doc Ophthalmol* 90:377-386
- Frennesson I, Nilsson S (1995) Effects of argon (green) laser soft drusen in early age-related maculopathy: a 6 months prospective study. *Br J Ophthalmol* 79:905-909
- Freund K, Yannuzzi L, Sorenson J (1993) Age-related macular degeneration and choroidal neovascularisation. *Am J Ophthalmol* 115:786-791
- Frey F (1999) Immunosuppressive drugs--useful confusion of the 20th century? [In German]. *Ther Umsch* 56:708-712
- Friedman D, O'Colmain B, Munoz B, Tomany S, McCarty C, de Jong P, Nemesure B, Mitchell P, Kempen J, Eye Diseases Prevalence Research Group (2004a)

- Prevalence of age-related macular degeneration in the United States. *Arch Ophthalmol* 122:564-572
- Friedman E, Krupsky S, Lane A, Oak S, Friedman E, Egan K, Gragoudas E (1995) Ocular blood flow velocity in age-related macular degeneration. *Ophthalmology* 102:640-646
- Friedman E, Rigas I, Makar M (2004b) The role of statins and hypertensives in the pathogenesis and management of AMD. 2004 Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO):Abstract 3127
- Friedman N, Haegerstrom-Portnoy G, Paul O, Jampolsky A (1994) Foveal vision function before and after fluorescein angiography. *Invest Ophthalmol Vis Sci* 35:3566-3570
- Friedrichson T, Kalbach H, Buck P, van Kuijk F (1995) Vitamin E in macular and peripheral tissues of the human eye. *Curr Eye Res* 14:693-701
- Frishman L, Saszik S, Harwerth R, Viswanathan S, Li Y, Smith E, 3rd., Robson J, Barnes G (2000) Effects of experimental glaucoma in macaques on the multifocal ERG: multifocal ERG in laser-induced glaucoma. *Doc Ophthalmol* 100:231-251
- Fujii G, De Juan EJ, Humayun M, Sunness J, Chang T, Rossi J (2003) Characteristics of visual loss by scanning laser ophthalmoscope microperimetry in eyes with subfoveal choroidal neovascularization secondary to age-related macular degeneration. *Am J Ophthalmol* 136:1067-1078
- Gale CR, Hall NF, Phillips DIW, Martyn CN (2003) Lutein and Zeaxanthin Status and Risk of Age-Related Macular Degeneration. *Invest. Ophthalmol. Vis. Sci.* 44:2461-2465
- Gao H, Hollyfield J (1992) Aging of the human retina. *Invest Ophthalmol Vis Sci* 33:1-17
- Gartner S, Henkind P (1981) Aging and degeneration of the human macula. 1. Outer nuclear layer and photoreceptors. *Br J Ophthalmol* 65:23-28
- Gass J (1967) Pathogenesis of disciform macular detachment of the neuroepithelium. 3. Senile disciform macular degeneration. *Am J Ophthalmol* 63:617-644

- Gass J (1972) Drusen and disciform macular detachment and degeneration. *Trans Am Ophth Soc* 70:409-436
- Gass J (1973) Drusen and disciform macular detachment and degeneration. *Arch Ophthalmol* 90:206-217
- Gass J, Jallow S, Davis B (1985) Adult vitelliform macular degeneration occurring in patients with basal laminar drusen. *Am J Ophthalmol* 99:445-459
- Gerth C, Garcia S, Ma L, Keltner J, Werner J (2002) Multifocal electroretinogram: age-related changes for different luminance levels. *Graefe's Arch Clin Exp Ophthalmol* 240:202-208
- Gerth C, Hause D, Delahunt P, Morse L, Werner J (2003) Assessment of multifocal electroretinogram abnormalities and their relation to morphologic characteristics with large drusen. *Arch Ophthalmol* 121:1404-1414
- Ghafour M, Allen D, Foulds W (1983) Common causes of blindness and visual handicap in the West of Scotland. *Br J Ophthalmol* 67:209-213
- Ghazi G, Jabbour N, Del la Cruz Z, Green W (2001) Clinicopathologic studies of age-related macular degeneration with classic subfoveal neovascularisation treated with photodynamic therapy. *Retina* 21:478-486
- Gillies M, Simpson J, Luo W, Penfold P, Hunyor A, Chua W, Mitchell P, Billson F (2003) A randomized clinical trial of a single dose of intravitreal triamcinolone acetonide for neovascular age-related macular degeneration: one-year results. *Arch Ophthalmol* 121:667-673
- Glaser B (1988) Extracellular modulating factors and the control of intraocular neovascularisation. *Arch Ophthalmol* 106:603-607
- Glaser B, Campochiaro P, Davis J, Sato M (1985) Retinal pigment epithelial cells release an inhibitor to neovascularisation. *Arch Ophthalmol* 103:1870-1875
- Goldsmith T (1994) Ultraviolet receptors and color vision: evolutionary implications and dissonance of paradigms. *Vision Res* 34:1479-1487
- Gonzalez P, Parks S, Keating D (2004) The effect of pupil size on the multifocal electroretinogram. 2004 Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO):Abstract 4246
- Gordon W, Casey D, Lukiw W, Bazan N (2002) DNA damage and repair in light induced photoreceptor degeneration. *Invest Ophthalmol Vis Sci* 43:3511-3521

- Gorin M, Breitner J, De Jong P, Hageman G, Klaver C, Kuehn M, Seddon J (1999) The genetics of age-related macular degeneration. *Mol Vis* 5:29
- Gorin M, Jackson K, Ferrell R, Sheffield V, Jacobson SG, Gass J, Mitchell E, Stone E (1995) A peripherin/retinal degeneration slow mutation (Pro-210-Arg) associated with macular and peripheral retinal degeneration. *Ophthalmology* 102:246-255
- Gouras P, Algvere P (1996) Retinal cell transplantation in the macula: new techniques. *Vision Res* 36:4121-4125
- Gouras P, Eggers H (1984) Herring's opponent colour channels do not exist in the primate retinogeniculate pathway. *Ophthalmic Res* 16:31-35
- Green W (1999) Histopathology of age-related macular degeneration. *Mol Vis* 5:27
- Green W, Enger C (1993) Age-related macular degeneration histopathological studies. The Lorenz E. Zimmermann Lecture. *Invest Ophthalmol Vis Sci* 100:1519-1535
- Greenstein V, Chen H, Hood D, Seiple W, Carr R (2000a) Retinal function in diabetic macular edema after focal laser photocoagulation. *Invest Ophthalmol Vis Sci* 41:3655-3664
- Greenstein V, Holopigian K, Hood D, Seiple W, Carr R (2000b) The nature and extent of retinal dysfunction associated with diabetic macular edema. *Invest Ophthalmol Vis Sci* 41:3643-3654
- Greenstein V, Holopigian K, Seiple W, Carr R, Hood D (2004) Atypical multifocal ERG responses in patients with diseases affecting the photoreceptors. *Vision Res* 44:2867-2874
- Greenstein V, Hood D, Carr R (1987) S cone pathway vulnerability: differential effects of retinitis pigmentosa and diabetes. *Invest Ophthalmol Vis Sci* 28:113
- Greenstein V, Hood D, Carr R (1989a) A comparison of S cone pathway sensitivity loss in patients with diabetes and retinitis pigmentosa. In Drum and G. Verriest (eds.), *Colour Vision Deficiencies IX*, Kluwer Academic Publishers, Dordrecht:233-241
- Greenstein V, Hood D, Ritch R, Steinberg R, Carr R (1989b) S (Blue) cone pathway vulnerability in retinitis pigmentosa, diabetes and glaucoma. *Invest Ophthalmol Vis Sci* 30:1732-1737

- Greenstein V, Thomas S, Blaustein H, Koenig K, Carr R (1993) Effects of early diabetic retinopathy on rod system sensitivity. *Optom Vis Sci* 37:1140-1148
- Gregor Z, Joffe L (1978) Senile macular changes in black African. *Br J Ophthalmol* 62:547-550
- Grindle C, Marshall J (1978) Aging changes in Bruch's membrane and their functional implications. *Trans Ophthalmol Soc UK* 98:172-175
- Grossniklaus H, Green W (1998) Histopathologic and ultrastructural findings of surgically excised choroidal neovascularisation. Submacular Surgery Trials Research Group. *Arch Ophthalmol* 116:745-749
- Grossniklaus H, Green W (2004) Choroidal neovascularisation. *Am J Ophthalmol* 137:496-503
- Grozdanic S, Sakaguchi D, Kwon Y, Kardon R, Sonea I (2003) Functional characterization of retina and optic nerve after acute ocular ischemia in rats. *Invest Ophthalmol Vis Sci* 44:2597-2605
- Grunwald J, Hariprasad S, DuPont J, Maguire M, Fine S, Brucker A, Maguire A, Ho A (1998) Foveal choroidal blood flow in age-related macular degeneration (AMD). *Invest Ophthalmol Vis Sci* 39:385-390
- Grunwald J, Metelitsina T, DuPont J, Ying G-S, Maguire M (2005) Reduced foveolar choroidal blood flow in eyes with increasing AMD severity. *Invest Ophthalmol Vis Sci* 46:1033-1038
- Guerne D, Tso M, Edward D, Ripps H (1991) Antiretinal antibodies in serum of patients with age-related macular degeneration. *Ophthalmology* 98:602-607
- Guymer R, Gros-Jendroska M, Owens S, Bird A, Fitzke F (1997) Laser treatment in subjects with high-risk clinical features of age-related macular degeneration. Posterior pole appearance and retinal function. *Arch Ophthalmol* 115:595-603
- Guymer R, Luthert P, Bird A (1998) Changes in Bruch's membrane and related structures with age. *Prog Ret Eye Res* 18:59-90
- Haegerstrom-Portnoy G (1988) Short-wavelength-sensitive-cone sensitivity loss with aging: a protective role for the macular pigment? *J Opt Soc Am A Opt Image Sci Vis* 5:2140-2144
- Haegerstrom-Portnoy G, Brabyn J, Schneck M, Jampolsky A (1997) The SKILL Card. An acuity test of reduced luminance and contrast. *Invest Ophthalmol Vis Sci* 38:207-218

- Haegerstrom-Portnoy G, Brown B (1989) Two-color increment thresholds in early age-related maculopathy. *Clin Vis Sci* 4:165-172
- Hageman G, Luthert P, Victor Chong N, Johnson L, Anderson D, Mullins R (2001) An integrated hypothesis that considers drusen as biomarkers of immune-mediated processes at the RPE-Bruch's membrane interface in aging and age-related macular degeneration. *Prog Retinal Eye Res* 20:705-732
- Haimovici R, Gantz D, Rumelt S (2001) The lipid composition of drusen, Bruch's membrane, and sclera by hot stage polarizing light microscopy. *Invest Ophthalmol Vis Sci* 42:1592-1599
- Haimovici R, Owens S, Fitzke F, Bird A (2002) Dark adaptation in age-related macular degeneration: relationship to the fellow eye. *Graefe's Arch Clin Exp Ophthalmol* 240:90-95
- Hall N, Gale C, Syddall H, Martyn C, Phillips D (2002) Relation between size at birth and risk of age-related macular degeneration. *Invest Ophthalmol Vis Sci* 43:3641-3645
- Hall N, Gale C, Syddall H, Phillips D, Martyn C (2001) Risk of macular degeneration in users of statins: cross sectional study. *BMJ* 323:375-376
- Hammond B, Johnson E, Russell R, Krinsky N, Yeum K, Edwards R, Snodderly D (1997) Dietary modification of human macular pigment density. *Invest Ophthalmol Vis Sci* 38:1795-1801
- Hammond B, Wooten B, Snodderly D (1996) Cigarette smoking and retinal carotenoids: implications for age-related macular degeneration. *Vis Res* 36:3003-3009
- Hammond C, Webster A, Snieder H, Bird A, Gilbert C, Spector T (2002) Genetic Influence on early age-related maculopathy. A twin study. *Ophthalmology* 109:730-763
- Han Y, Bearse MJ, Schneck M, Barez S, Jacobsen C, Adams A (2004a) Towards optimal filtering of 'standard' multifocal electroretinogram (mfERG) recordings: findings in normal and diabetic subjects. *Br J Ophthalmol* 88:543-550
- Han Y, Bearse MJ, Schneck M, Barez S, Jacobson C, Adams A (2004b) Multifocal electroretinogram delays predict sites of subsequent diabetic retinopathy. *Invest Ophthalmol Vis Sci* 45:948-954

- Handa J, Reiser K, Matsunaga H, Hjelmeland L (1998) The advanced glycation endproducts pentosidine induces the expression of PDGF-B in human retinal pigment epithelium cells. *Exp Eye Res* 66:411-419
- Handa J, Verzijl N, H. M, Aotaki-Keen A, Luttj G, Koppele J, Miyata T, Hjelmeland L (1999) Increase in the advanced glycation end product pentosidine in Bruch's membrane with age. *Invest Ophthalmol Vis Sci* 40:775-779
- Hangai M, Murata T, Miyawaki N, Spee C, Lim J, He S, Hinton D, Ryan S (2001) Angiopoietin-1 upregulation by vascular endothelial growth factor in human retinal pigment epithelial cells. *Invest Ophthalmol Vis Sci* 42:1617-1625
- Hankins M, Jones R, Ruddock K (1998) Diurnal variation in the b-wave implicit time of the human electroretinogram. *Vis Neurosci* 15:55-67
- Harada K, Friedman M, Lopez J, Wang S, Li J, Prasa P, Pearlman J, Edelman E, Sellke F, Simons M (1996) Vascular endothelial growth factor administration in chronic myocardial ischemia. *Am J Physiol* 270:1791-1802
- Hare W, Ton H (2002) Effects of APB, PDA, and TTX on ERG responses recorded using both multifocal and conventional methods in monkey. Effects of APB, PDA, and TTX on monkey ERG responses. *Doc Ophthalmol* 105:189-222
- Harris A, Chung H, Ciulla T, Kagemann L (1999) Progress in measurement of ocular blood flow and relevance to our understanding of glaucoma and age-related macular degeneration. *Prog Ret Eye Res* 18:669-687
- Hart W (1987) Acquired dyschromatopsias. *Surv Ophthalmol* 32:10-31
- Hart W, Burde R (1983) Three-dimensional topography of the central visual field. Sparing of foveal sensitivity in macular disease. *Ophthalmology* 90:1028-1038
- Hasegawa T, Takagi C, Usui S, Takada R, Abe H (2000) Waveform changes of the first-order multifocal electroretinogram in patients with glaucoma. *Invest Ophthalmol Vis Sci* 41:1570-1579
- Hayasaka S (1989) Aging changes in lipofuscin, lysosomes and melanin in the macular area of human retina and choroid. *Jpn J Ophthalmol* 33:33-42
- Hayes K (1978) Retinal degeneration in monkeys induced by deficiencies of vitamin E or A. *Invest Ophthalmol Vis Sci* 13:499-510
- Hayreh S, Gartner S (1990) In vivo choroidal circulation and its watershed zones. *Eye* 4:273-289

- Heckenlively J, Aptsiauri N, Nusinowitz S, Peng C, Hargrave P (1996) Investigations of antiretinal antibodies in pigmentary retinopathy and other retinal degenerations. *Trans Am Ophthalmol Soc* 94:179-200
- Hee M, Bauman C, Puliafito C, Duker J, Reichel E, Wilkins J, Coker J, Schuman J, Swanson E, Fujimoto J (1996) Optical coherence tomography of age-related macular degeneration and choroidal neovascularization. *Ophthalmology* 103:1260-1270
- Hee M, Izatt J, Swanson E, Huang D, Schuman J, Lin C, Puliafito C, Fujimoto J (1995) Optical coherence tomography of the human retina. *Arch Ophthalmol* 113:325-332
- Heiba I, Elston R, Klein B, Klein R (1994) Sibling correlations and segregation analysis of age-related maculopathy: The Beaver Dam Eye Study. *Gen Epidemiol* 11:51-67
- Heinemann-Vernaleken B, Palmowski A, Allgayer R (2000) The effect of time of day and repeat reliability on the fast flicker multifocal ERG. *Doc Ophthalmol* 101:247-255
- Heinemann-Vernaleken B, Palmowski A, Allgayer R, Ruprecht K (2001) Comparison of different high resolution multifocal electroretinogram recordings in patients with age-related maculopathy. *Graefe's Arch Clin Exp Ophthalmol* 239:556-561
- Hendry S, Calkins D (1998) Neuronal chemistry and functional organisation in the primate visual system. *Trends Neurosci* 21:344-349
- Hendry S, Yoshioka T (1994) A neurochemically distinct third channel in the macaque dorsal lateral geniculate nucleus. *Science* 264:575-577
- Henkes H (1954) ERG in circulatory disturbances of the retina. III. ERG in cases of senile degeneration of the macular area. *Arch Ophthalmol* 51:54-66
- Heuberger R, Mares-Perlmann J, Klein R, Klein B, Millen A, Palta M (2001) Relationship of dietary fat to age-related maculopathy in the third national health and nutrition examination survey. *Arch Ophthalmol* 119:1833-1838
- Hicks D, Sahel JA (1999) The implications of rod-dependent cone survival for basic and clinical research. *Invest Ophthalmol Vis Sci* 40:3071-3074
- Hinton D, He S, Lopez P (1998) Apoptosis in surgically excised choroidal neovascular membranes in age-related macular degeneration. *Arch Ophthalmol* 116:203-209

- Hogan M, Alvarado J (1967) Studies on the human macula. IV. Aging changes in Bruch's membrane. *Arch Ophthalmol* 77:410-420
- Holopigian K, Greenstein V, Seiple W, Hood D, Carr R (1997a) Evidence for photoreceptor changes in patients with diabetic retinopathy. *Invest Ophthalmol Vis Sci* 38:2355-2365
- Holopigian K, Seiple W, Greenstein V, Hood D, Carr R (2001) Local cone and rod system function in patients with retinitis pigmentosa. *Invest Ophthalmol Vis Sci* 42:779-788
- Holopigian K, Seiple W, Greenstein V, Hood D, Carr R (2002) Local cone and rod system function in progressive cone dystrophy. *Invest Ophthalmol Vis Sci* 43:2364-2373
- Holopigian K, Seiple W, Greenstein V, Kim D, Carr R (1997b) Relative effects of aging and age-related macular degeneration on peripheral visual function. *Optom Vis Sci* 74:152-159
- Holz F, Bellmann C, Dithmar S, Rohrschneider K, Burk R, Völcker H (1997) Confocal scanning laser fluorescein and indocyanine green angiography [in German]. *Ophthalmologie* 94:348-353
- Holz F, Bellmann C, Margaritidis M, Schutt F, Otto T, Völcker H (1999a) Patterns of increased in vivo fundus autofluorescence in the junctional zone of geographic atrophy of the retinal pigment epithelium associated with age-related macular degeneration. *Graefe's Arch Clin Exp Ophthalmol* 237:145-152
- Holz F, Bellmann C, Staudt S, Schutt F, Völcker H (2001) Fundus autofluorescence and development of geographic atrophy in age-related macular degeneration. *Invest Ophthalmol Vis Sci* 42:1051-1056
- Holz F, Pauleikhoff D, Klein R, Bird A (2004) Pathogenesis of lesions in late age-related macular disease. *Am J Ophthalmol* 137:504-510
- Holz F, Schuett F, Kopitz J, Graig E, FE. K, Völcker H, Cantz M (1999b) Inhibition of lysosomal degradative functions in RPE cells by a retinoid component of Lipofuscin. *Invest Ophthalmol Vis Sci* 40:737-743
- Holz F, Sheraidah G, Pauleikhoff D, AC B (1994a) Analysis of lipid deposits extracted from human macular and peripheral Bruch's membrane. *Arch Ophthalmol* 112:402-406

- Holz F, TJ. W, Piguet B, M. G-J, Well J, Minassian D, IH. C, Bird A (1994b)
 Bilateral macular drusen in age-related macular degeneration. Prognosis and
 risk factors. *Ophthalmology* 101:1522-1528
- Hood D (2000) Assessing retinal function with the multifocal technique. *Prog Ret
 Eye Res* 19:607-646
- Hood D, Benimoff N, Greenstein V (1984) The response range of the blue-cone
 pathways: a source of vulnerability to disease. *Invest Ophthalmol Vis Sci*
 25:864-867
- Hood D, Birch D (1996) Beta wave of the scotopic (rod) ERG as a measure of the
 activity of human on-bipolar cells. *J Opt Soc Am A* 13:623-633
- Hood D, Frishman L, Robson A, Shady S, Ahmed J, Viswanathan S (1999a) A
 frequency analysis of regional variation in the contribution from action
 potentials to the primate multifocal ERG. In: *Vision Science and its
 Applications*, OSA Tech Dig Ser, Optical Society of America, Washington,
 DC:56-59
- Hood D, Frishman L, Saszik S, Viswanathan S (2002) Retinal origins of the primate
 multifocal ERG. Implication for the human response. *Invest Ophthalmol Vis
 Sci* 43:1673-1685
- Hood D, Frishman L, Viswanathan S, Robson J, Ahmed J (1999b) Evidence for a
 ganglion cell contribution to the primate electroretinogram (ERG): effects of
 TTX on the multifocal ERG in macaque. *Vis Neurosci* 16:411-416
- Hood D, Greenstein V (1988) Blue (S) cone pathway vulnerability: a test of a fragile
 receptor hypothesis. *Appl Opt* 27:1025-1029
- Hood D, Greenstein V, Frishman L, Holopigian K, Viswanathan S, Seiple W,
 Ahmed J, Robson J (1999c) Identifying inner retinal contributions to the
 human multifocal electroretinogram. *Vision Res* 39:2285-2291
- Hood D, Greenstein V, Holopigian K, Bauer R, Firoz B, Liebmann J, Odel J, Ritch R
 (2000) An attempt to detect glaucomatous damage to the inner retina with the
 multifocal ERG. *Invest Ophthalmol Vis Sci* 41:1597-1603
- Hood D, Holopigian K, Greenstein V, Seiple W, Li J, Sutter E, Carr R (1998a)
 Assessment of local retinal function in patients with retinitis pigmentosa
 using the multi-focal ERG technique. *Vision Res* 38:163-179

- Hood D, Li J (1997) A technique for measuring individual multifocal ERG records. *Noninvasive Assessment of the Visual System: Trends in Optics and Photonics* 11:33-41
- Hood D, Seiple W, Holopigian K, Greenstein V (1997) A comparison of the components of the multifocal and full-field ERGs. *Vis Neurosci* 14:533-544
- Hood D, Wladis E, Shady S, Holopigian K, Li J, Seiple W (1998b) Multifocal rod electroretinograms. *Invest Ophthalmol Vis Sci* 39:1152-1161
- Hood D, Zhang X (2000) Multifocal ERG and VEP responses and visual fields: comparing disease related changes. *Doc Ophthalmol* 100:115-137
- Hopley C, Salkeld G, Mitchell P (2004a) Cost utility of photodynamic therapy for predominantly classic neovascular age related macular degeneration. *Br J Ophthalmol* 88:982-987
- Hopley C, Salkeld G, Wang J, Mitchell E (2004b) Cost utility of screening and treatment for early age related macular degeneration with zinc and antioxidants. *Br J Ophthalmol* 88:450-454
- Horiguchi M, Miyake Y, Fujii G (1991) Human focal rod ERG. 1991 Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO) 32:926
- Horiguchi M, Suzuki S, Kondo M, Tanikawa A, Miyake Y (1998) Effect of glutamate analogues and inhibitory neurotransmitters on the electroretinograms elicited by random sequence stimuli in rabbits. *Invest Ophthalmol Vis Sci*:2171-2176
- Horio N, Horiguchi M (2004) Effect on visual outcome after macular hole surgery when staining the internal limiting membrane with indocyanine green dye. *Arch Ophthalmol* 122:992-996
- Huang S, Wu D, Jiang F, Ma J, Wu L, Liang J, Luo G (2000) The multifocal electroretinogram in age-related maculopathies. *Doc Ophthalmol* 101:115-124
- Hvarfner C, Andreasson S, Larsson J (2003) Multifocal electroretinogram in branch retinal vein occlusion. *Am J Ophthalmol* 136:1163-1165
- Hyman L (1992) Epidemiology of AMD. In Hampton RG, Nelson PT (eds)., *Age Related Macular Degeneration: Principles and Practice*. New York: Raven Press:1-35

- Hyman L, Schachat A, He Q, Leske M (2000) Hypertension, cardiovascular disease, and age-related macular degeneration: Age-Related Macular Degeneration Risk Factors Study Group. *Arch Ophthalmol* 117:351-358
- Ikram M, van Leeuwen R, Vingerling J, Hofman A, de Jong P (2003) Relationship between refraction and prevalent as well as incident age-related maculopathy: The Rotterdam Study. *Invest Ophthalmol Vis Sci* 44:3778-3782
- Jablon E, Lavaque A, Chaudhry N, Tom D, Alfaro D (2004) Combined transpupillary thermotherapy enhanced with indocyanine green and intravitreal triamcinolone acetate for choroidal neovascularisation in age-related macular degeneration. 2004 Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO):Abstract 5126
- Jackson C, McGwin G, Phillips J, Klein R, Owsley C (2004) Impact of aging and age-related maculopathy on activation of the a-wave of the rod-mediated electroretinogram. *Invest Ophthalmol Vis Sci* 45:3271-3278
- Jackson G, De Leon Ortega J, Girkin C, Rosenstil C, Owsley C (2002a) Aging-related changes in the multifocal electroretinogram. *Opt Soc Am* 19:185-189
- Jackson G, Owsley C (2000) Scotopic sensitivity during adulthood. *Vision Res* 40:2467-2473
- Jackson G, Owsley C, Cordle E, Finley C (1998) Aging and scotopic sensitivity. *Vision Res* 38:3655-3662
- Jackson G, Owsley C, Curcio C (2002b) Photoreceptor degeneration and dysfunction in aging and age-related maculopathy. *Ageing Research Reviews* 1:381-396
- Jackson G, Owsley C, McGwin G (1999) Aging and dark adaptation. *Vision Res* 39:3975-3982
- Jacobson S, Cideciyan A, Regunath G, Rodriguez F, Vandenberg K, Sheffield V, Stone E (1995) Night blindness in Sorsby's fundus dystrophy reversed by vitamin A. *Nat Genet* 11:27-32
- Jahn C, Wuestenmayer H, Brinkmann C, Trautmann S, Moessner A, Schnurrbusch U, Wolf S (2004) Macular pigment density subject to different stages of age-related maculopathy. 2004 Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO):Abstract 2968
- Jampol L (2003) AREDS-two years later. *Arch Ophthalmol* 121:1634-1635

- Jiang L, Jin C, Huang S, Wu D, Wu L (2003) The changes of multifocal electroretinography in the early stage of photodynamic therapy for choroidal neovascularisation. *Doc Ophthalmol* 107:165-170
- Johnson A, Lutty G, McLeod D, Otsuji T, Flower R, Sandagar G, Alexander T, Steidl S, Hansen B (2005) Ocular structure and function in an aged monkey with spontaneous diabetes mellitus. *Exp Eye Res* 80:37-42
- Johnson L, Leitner W, Staples M, Anderson D (1999) Complement activation and inflammatory processes in drusen formation and age-related macular degeneration. *Exp Eye Res* 73:887-896
- Johnson L, Ozaki S, Staples M, Erickson P, Anderson D (2000) A potential role for immune complex pathogenesis in drusen formation. *Exp Eye Res* 70:441-449
- Johnson P, Lewis G, Talaga K, Brown M, Kappel P, Fisher S, Anderson D, Johnson L (2003) Drusen-associated degeneration in the retina. *Invest Ophthalmol Vis Sci* 44:4481-4488
- Jonas J (2004) Intravitreal triamcinolone acetonide as treatment for extensive exudative retinal detachment. *Br J Ophthalmol* 88:587-588
- Jonas J, Kreissig I, Degenring R (2003) Intraocular pressure after intravitreal injection of triamcinolone acetonide. *Br J Ophthalmol* 87:24-27
- Jones R, King-Smith P, Loffing D, Gaynier F (1986) Stray light contributions to the focal electroretinogram (ERG). *Clin Vis Sci* 1:153-160
- Journee-de Korver K, Oosterhuis J, de Wolff-Rouendaal D, Kemme H (1997) Histopathologic findings in human choroidal melanomas after transpupillary thermotherapy. *Br J Ophthalmol* 81:234-239
- Jurklies B, Sutter E (2002) Reply to G. Rudolph and P. Kalpadakis: the role of fixation for reliable mfERG results. *Graefe's Arch Clin Exp Ophthalmol* 240:876-877
- Jurklies B, Weismann M, Bornfeld N (1999) Multifocal electroretinogram in age-related macular degeneration-changes in amplitude of the 1st-order kernel. 1999 Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO) 40:Abstract 1668
- Jurklies B, Weismann M, Bornfeld N (2000) Multifocal ERG in age-related macular degeneration with hard and soft drusen. 2000 Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO) 41:Abstract 4749

- Jurklies B, Weismann M, Sutter E, Bornfeld N (2002) Monitoring retinal function in neovascular maculopathy using multifocal electroretinography-early and long-term correlation with clinical findings. *Graefe's Arch Clin Exp Ophthalmol* 240:244-264
- Kaiser P, Boynton R (1996) In: Human color vision. Second Edition. Kaiser P and Boynton M, (eds.). Optical Society of America
- Kaiser P, Verteporfin In Occult (VIO) Study Group (2004) Verteporfin in occult (VIO): the design of a phase III controlled trial of verteporfin therapy for occult with no classic subfoveal CNV secondary to AMD. 2004 Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO):Abstract 2276
- Kalpadakis P, Rudolph G (2003) Multifocal ERG with the scanning laser ophthalmoscope: query on the ideal configuration for attaining high resolution and result stability. *Graefe's Arch Clin Exp Ophthalmol* 241:522
- Kaplan E, Shapley R (1986) The primate retina contains two types of ganglion cells, with high and low contrast sensitivity. *Proc Natl Acad Sci USA* 83:2755-2757
- Karcioglu Z (1982) Zinc in the eye. *Surv Ophthalmol* 27:114-122
- Katz J, Sommer A (1986) Asymmetry and variation in the normal hill of vision. *Arch Ophthalmol* 104:65-68
- Keating D, Parks S, Evans A (2000) Technical aspects of multifocal ERG recording. *Doc Ophthalmol* 100:77-98
- Keating D, Parks S, Evans A, Williamson T, Elliot A, Jay J (1997) The effect of filter bandwidth on the multifocal electroretinogram. *Doc Ophthalmol* 92:291-300
- Keating D, Parks S, Malloch C, Evans A (2001) A comparison of CRT and digital stimulus delivery methods in the multifocal ERG. *Doc Ophthalmol* 102:95-114
- Keating D, Parks S, Smith D, Evans A (2002) The multifocal ERG: unmasked by selective cross-correlation. *Vision Res* 42:2959-2968
- Keating D, Parks S, Williamson T, Evans A, Jay J, Elliot A (1996) The effect of pupil dilation, retinal blur and filter bandwidth on the multi-focal ERG. *Invest Ophthalmol Vis Sci* 37(suppl):346

- Kellner U, Kraus H, Foerster M (2000) Multifocal ERG in chloroquine retinopathy: regional variance of retinal dysfunction. *Graefe's Arch Clin Exp Ophthalmol* 238:94-97
- Kemp C, Jacobson S, Faulkner D, Walt R (1988) Visual function and rhodopsin level in humans with vitamin A deficiency. *Exp Eye Res* 46:185-197
- Kenyon K, Maumenee A, Ryan S, Whitmore P, Green W (1985) Diffuse drusen and associate complication. *Am J Ophthalmol* 100:119-128
- Keys S, Zimmerman W (1999) Antioxidant activity of retinol, glutathione, and taurine in bovine photoreceptor cell membranes. *Exp Eye Res* 68:693-702
- Khachik F, Bernstein P, Garland D (1997) Identification of lutein and zeaxanthin oxidation products in human and monkey retinas. *Invest Ophthalmol Vis Sci* 38:1802-1811
- Killingsworth M, Sarks J, Sarks S (1990) Macrophages related to Bruch's membrane in age-related macular degeneration. *Eye* 4:613-621
- Kiryu J, Asrani S, Shahidi M, Mori M, Zeimer R (1995) Local response of the primate retinal microcirculation to increased metabolic demand induced by flicker. *Invest Ophthalmol Vis Sci* 36:1240-1246
- Klaver C, Assink J, van Leeuwen R, Wolfs R, Vingerling J, Stijnen T, Hofman A, de Jong P (2000) Incidence and progression rates of age-related maculopathy: the Rotterdam Study. *Invest Ophthalmol Vis Sci* 42:2237-2241
- Klaver C, Ott A, Hofman A, Assink J, Breteler M, de Jong P (1999) Is age-related maculopathy associated with Alzheimer's Disease? The Rotterdam Study. *Am J Epidemiol.* 150:963-968
- Klaver C, Wolfs R, Assink J, van Duijn C, Hofman A, de Jong P (1998) Genetic risk of age-related maculopathy. Population-based family aggregation study. *Arch Ophthalmol* 116:1646-1651
- Klein B, Klein R, Lee K (2000) Reproductive exposure, incident age-related cataracts, and age-related maculopathy in women: the Beaver Dam Eye Study. *Am J Ophthalmol* 130:322-326
- Klein M, Mauldin W, Stoubos V (1994) Hereditary and age-related macular degeneration. Observation in monozygotic twins. *Arch Ophthalmol* 112:932-937
- Klein R, Davis M, Magli Y, Segal P, Klein B, Hubbard L (1991) The Wisconsin Age-Related Maculopathy Grading System. *Ophthalmology* 131:167-175

- Klein R, Klein B, Cruickshanks K (1999a) The prevalence of age-related maculopathy by geographic region and ethnicity. *Prog Retin Eye Res* 18:371-389
- Klein R, Klein B, Jensen S (1997a) The relation of cardiovascular disease and its risk factors to the 5-year incidence of age-related maculopathy: the Beaver Dam Eye Study. *Ophthalmology* 104:1804-1812
- Klein R, Klein B, Jensen S, Mares-Perlman J, Cruickshanks K, Palta M (1999b) Age-related maculopathy in a multiracial United States population: the National Health and Nutrition Examination Survey III. *Ophthalmology* 106:1056-1065
- Klein R, Klein B, Jensen S, Meuer S (1997b) The five-year incidence and progression of age-related maculopathy: The Beaver Dam Eye Study. *Ophthalmology* 104:7-21
- Klein R, Klein B, Linton K (1992) Prevalence of age-related maculopathy. *Ophthalmology* 99:933-943
- Klein R, Klein B, Linton K, DeMets D (1993) The Beaver Dam Eye Study: the relation of age-related maculopathy to smoking. *Am J Epidemiol.* 137:190-200
- Klein R, Klein B, Marino E, Kuller L, Furberg C, Burke G, Hubbard L (2003a) Early age-related maculopathy in the cardiovascular health study. *Ophthalmology* 110:25-33
- Klein R, Klein B, Moss S (1998) Relation of smoking to the incidence of age-related maculopathy. The Beaver Dam Eye Study. *Am J Epidemiol.* 147:103-110
- Klein R, Klein B, Tomany S, Cruickshanks K (2003b) The association of cardiovascular disease with long term incidence of age-related maculopathy. *Ophthalmology* 110:636-643
- Klein R, Klein B, Tomany S, Cruickshanks K (2003) Association of emphysema, gout, and inflammatory markers with long-term incidence of age-related maculopathy. *Arch Ophthalmol* 121:674-678
- Klein R, Klein B, Tomany S, Danforth L, Cruickshanks K (2003c) Relation of statin use to the 5-year incidence and progression of age-related maculopathy. *Arch Ophthalmol* 121:1151-1155

- Klein R, Klein B, Tomany S, Meuer S, Huang G (2002) Ten-years incidence and progression of age-related maculopathy: The Beaver Dam Eye study. *Ophthalmology* 109:1767-1779
- Klein R, Peto T, Bird A, VanNewkirk M (2004) The epidemiology of age-related macular degeneration. *Am J Ophthalmol* 137:486-495
- Klein R, Rowland M, Harris M (1995) Racial/ethnic differences in age-related maculopathy. Third National Health and Nutrition Examination Survey. *Ophthalmology* 102:371-381
- Kleiner R, Enger C, Alexander M, Fine S (1988) Contrast sensitivity in age-related macular degeneration. *Arch Ophthalmol* 106:55-57
- Kliffen M, Sharma H, Mooy C, Kerkvliet S, de Jong P (1997) Increased expression of angiogenic growth factors in age-related maculopathy. *Br J Ophthalmol* 81:154-162
- Knight R, Buck S, Fowler G, Nguyen A (1998) Rods affect S-cone discrimination on Farnsworth-Munsell 100-hue test. *Vis Res* 38:3477-3481
- Koh H-H, Murray I, Nolan D, Carden D, Feather D, Beatty S (2004) Plasma and macular response to lutein supplement in subjects with and without age-related maculopathy: a pilot study. *Exp Eye Res* 79:21-27
- Kolb H, Framiglietti E (1974) Rod and cone pathways in the inner plexiform layer of the cat retina. *Science* 186:47-49
- Kolb H, Nelson R (1983) Rod pathways in the retina of the cat. *Vision Res* 23:301-312
- Kondo M, Miyake Y, Horiguchi M, Suzuki S (1997) Recording multifocal electroretinograms with fundus monitoring. *Invest Ophthalmol Vis Sci* 38:1049-1052
- Kondo M, Miyake Y, Horiguchi M, Suzuki S, Tanikawa A (1995) Clinical evaluation of multifocal electroretinogram. *Invest Ophthalmol Vis Sci* 36:2146-2150
- Kondo M, Miyake Y, Horiguchi M, Suzuki S, Tanikawa A (1998) Recording multifocal electroretinogram on and off responses in humans. *Invest Ophthalmol Vis Sci* 39:574-580
- Kondo M, Miyake Y, Kondo M, Tanikawa A, Suzuki S, Horiguchi M, Terasaki H (2001) Multifocal ERG findings in complete type congenital stationary night blindness. *Invest Ophthalmol Vis Sci* 42:1342-1348

- Kornzweig A (1977) Changes of the choriocapillaris associated with senile macular degeneration. *Ann Ophthalmol* 9:753-764
- Kretschmann U, Bock M, Gockeln R, Zrenner E (2000) Clinical applications of multifocal electroretinography. *Doc Ophthalmol* 100:99-113
- Kretschmann U, Gendo K, Seeliger MW, Zrenner E (1997) Multifocal ERG recording by the VERIS technique and its clinical applications. *Dev Ophthalmol* 29:8-14
- Kretschmann U, Schlote T, Stuebiger N, Gendo K, Hipp E, Zrenner E (1998a) Multifocal electroretinography in acquired macular dysfunction [in German]. *Klin Monatsbl Augenheilkd* 212:93-100
- Kretschmann U, Seeliger M, Ruether K, Usui T, Zrenner E (1998b) Spatial cone activity distribution in diseases of the posterior pole determined by multifocal electroretinography. *Vision Res* 38:3817-3828
- Kretschmann U, Seeliger M, R  ther K, Usui T, Apfelstedt-Sylla E, Zrenner E (1998c) Multifocal electroretinography in patients with Stargardt's macular dystrophy. *Br J Ophthalmol* 82:267-275
- Krinsky N (1979) Carotenoid protection against oxidation. *Pure Appl Chem* 51:649-660
- Krzystolik M, Afshari M, Adamis A, Gaudreault J, Gragoudas E, Michaud N, Li W, Connolly E, O'Neill C, Miller J (2002) Prevention of experimental choroidal neovascularization with intravitreal anti-vascular endothelial growth factor antibody fragment. *Arch Ophthalmol* 120:338-406
- Kurtenbach A, Langrova H, Zrenner E (2000) Multifocal oscillatory potentials in type1 diabetes without retinopathy. *Invest Ophthalmol Vis Sci* 41:3234-3241
- Kvanta A (1995) Expression and regulation of vascular endothelial growth in choroidal fibroblasts. *Curr Eye Res* 14:1015-1020
- Kvanta A, Algvere P, Berglin L, Seregard S (1996) Subfoveal fibrovascular membranes in age-related macular degeneration express vascular endothelial growth factor. *Invest Ophthalmol Vis Sci* 37:1929-1934
- Lai T, Chan W-M, Lam D (2004) Transient reduction in retinal function revealed by multifocal electroretinogram after photodynamic therapy. *Am J Ophthalmol* 137:826-833
- Lamb T (1981) The involvement of rod photoreceptors in dark adaptation. *Vis Res* 21:2131-2139

- Lamb T, Pugh E (2004) Dark adaptation and the retinoid cycle of vision. *Prog Ret Eye Res* 23:307-380
- Lambert H, Capone AJ, Aaberg T, Sternberg PJ, Mandell B, Lopez P (1992) Surgical excision of subfoveal neovascular membranes in age-related macular degeneration. *Am J Ophthalmol* 113:257-262
- Lambert V, Munaut C, Carmeliet P, Gerard R, Declerck P, Gils A, Claes C, Foidart J, Noel A, Rakic J (2003) Dose-dependent modulation of choroidal neovascularization by plasminogen activator inhibitor type I: implications for clinical trials. *Invest Ophthalmol Vis Sci* 44:2791-2797
- Lamontagne N (2002) Raman techniques may detect macular degeneration early. *Biophotonics International* 9:38
- Landrum J, Bone R, Joa H, Kilburn M, Moore L, Sprague K (1997) A one year study of the macular pigment: the effect of 140 days of a lutein supplement. *Exp Eye Res* 65:57-62
- Lanthony P (1978) The desaturated panel D-15. *Doc Ophthalmol* 46:185-189
- LaVail M, Unoki K, Yasumura D, Matthes M, Yancopoulos G, Steinberg R (1992) Multiple growth factors, cytokines and neurotrophins rescue photoreceptors from damaging effects of constant light. *Proc Natl Acad Sci USA* 89:11249-11253
- Leibowitz H, Krueger D, Maunder L, Milton R, Kini M, Kahn H, Nickerson R, Pool J, Colton T, Ganley J, Loewenstein J, Dawber T (1984) The Framingham Eye Study monograph: an ophthalmological and epidemiological study of cataract, glaucoma, diabetic retinopathy, macular degeneration, and visual acuity in a general population of 2631 adults, 1973-1975. *Surv Ophthalmol* 25:333-610
- Leibrock C, Reuter T, Lamb T (1998) Molecular basis of dark adaptation in rod photoreceptors. *Eye*
- Li J, Tso M, Lam T (2001) Reduced amplitude and delayed latency in foveal response of multifocal electroretinogram in early age related macular degeneration. *Br J Ophthalmol* 85:287-290
- Li W, Yanoff M, Li Y, He Z (1999) Artificial senescence of bovine retinal pigment epithelial cells induced by near-red ultraviolet in vitro. *Mech Ageing Dev* 110:137-155

- Liles M, Newsome D, Oliver P (1991) Antioxidant enzymes in the aging human retinal pigment epithelium. *Arch Ophthalmol* 109:1285-1288
- Linsenmeier R, Padnick-Silver L (2000) Metabolic dependence of photoreceptors on the choroid in the normal and detached retina. *Invest Ophthalmol Vis Sci* 41:3117-3123
- Lip P, Blann A, Hope-Ross M, Gibson J, Lip G (2001) Age-related macular degeneration is associated with increased vascular growth factor, hemorheology and endothelial dysfunction. *Ophthalmology* 108:705-710
- Little H, Showman J, Brown B (1997) A pilot randomized controlled study on the effect of laser photocoagulation of confluent soft macular drusen. *Ophthalmology* 104:623-631
- Liu C, Li Y, Peng M, Laties A, Wen R (1999) Activation of caspase-3 in the retina of transgenic rats with the rhodopsin mutation s334ter during photoreceptor degeneration. *J Neurosci* 19:4778-4785
- Lopez P, Sippy B, Lambert H, Thatch A, Hinton D (1996) Transdifferentiated retinal pigment epithelial cells are immunoreactive for vascular endothelial growth factor in surgically excised age-related macular degeneration-related choroidal neovascular membranes. *Invest Ophthalmol Vis Sci* 37:855-868
- Lovie-Kitchin J (1985) *Senile macular degeneration: management and rehabilitation/* Jan E. Lovie-Kitchin, Kenneth J. Bowman. Boston: Butterworth Publishers.
- Lovie-Kitchin J, Brown B (1986) Reaction time in age-related maculopathy. *Am J Optom & Physiol Optics* 3:366-371
- Lovie-Kitchin J, Brown B (2000) Repeatability and intercorrelations of standard vision tests as a function of age. *Optom Vis Sci* 77:412-420
- Machemer R, Steinhorst U (1993) Retinal separation, retinotomy and macular relocation. A surgical approach for age-related macular degeneration. *Graefe's Arch Clin Exp Ophthalmol* 231:635-641
- Mackay A, Brown M, Hagan R, Grierson I, Wong S, Harding S (2004) Multifocal electroretinography deficits in neovascular age related macular degeneration. 2004 Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO):Abstract 3117
- Macular Photocoagulation Study Group (1982) Argon laser photocoagulation for senile macular degeneration. *Arch Ophthalmol* 100:912-918

- Macular Photocoagulation Study Group (1986) Argon laser photocoagulation for neovascular maculopathy. Three-year result from randomized clinical trials. Arch Ophthalmol 104:694-701
- Macular Photocoagulation Study Group (1990a) Krypton laser photocoagulation for neovascular lesions of age-related macular degeneration. Results of a randomized clinical trial. Arch Ophthalmol 108:816-824
- Macular Photocoagulation Study Group (1990b) Persistent and recurrent neovascularisation after krypton laser photocoagulation for neovascular lesions of age-related macular degeneration. Arch Ophthalmol 108:825-831
- Macular Photocoagulation Study Group (1991a) Laser photocoagulation of subfoveal neovascular lesions in age-related macular degeneration: results of a randomized clinical trial. Arch Ophthalmol 109:1220-1231
- Macular Photocoagulation Study Group (1991b) Subfoveal neovascular lesions in age-related macular degeneration. Guidelines for evaluation and treatment in the macular photocoagulation study. Arch Ophthalmol 109:1242-1257
- Macular Photocoagulation Study Group (1993) Laser photocoagulation of subfoveal neovascular lesions in age-related macular degeneration. Updated findings from two clinical trials. Arch Ophthalmol 111:1200-1209
- Macular Photocoagulation Study Group (1994a) Laser photocoagulation for juxtafoveal choroidal neovascularisation. Five-year results from randomized clinical trials. Arch Ophthalmol 112:500-509
- Macular Photocoagulation Study Group (1994b) Persistent and recurrent neovascularization after laser photocoagulation for subfoveal choroidal neovascularization of age-related macular degeneration. Arch Ophthalmol 112:489-499
- Macular Photocoagulation Study Group (1997) Risk factors for choroidal neovascularisation in the second eye of patients with juxtafoveal or subfoveal choroidal neovascularisation secondary to age-related macular degeneration. Arch Ophthalmol 115:741-747
- Marcus M, Merin S, Wolf M, Feinsod M (1983) Electrophysiological tests in assessment of senile macular degeneration. Ann Ophthalmol 15:235-238
- Mares-Perlman J, Brady W, Klein R, Klein B, VandenLangenberg G, Palta M (1995) Dietary fat and age-related maculopathy. Arch Ophthalmol 113:743-748

- Mares-Perlman J, Fisher A, Klein R, Palta M, Block G, Millen A, Wright J (2001) Lutein and zeaxanthin in the diet and serum and their relation to age-related maculopathy in the third national health and nutrition examination survey. *Am J Epidemiol* 153:424-432
- Mares-Perlman J, Klein R, Klein B, Greger J, Brady W, Palta M, Ritter L (1996) Association of zinc and antioxidant nutrients with age-related maculopathy. *Arch Ophthalmol* 114:991-997
- Marmor M (2002) "Do you, doctor, take the mfERG...for better or for worse?" *Graefe's Arch Clin Exp Ophthalmol* 240:241-243
- Marmor M, Hood D, Keating D, Kondo M, Seeliger M, Miyake Y (2003) Guidelines for basic multifocal electroretinography (mfERG). *Doc Ophthalmol* 106:105-115
- Marmor M, Tan F (1999) Central serous chorioretinopathy: bilateral multifocal abnormalities. *Arch Ophthalmol* 117:184-188
- Marre E, Marre M (1972) The influence of the three color vision-mechanisms on the spectral sensitivity of the fovea. *Mod Probl Ophthalmol* 11:219-223
- Marshall G, Konstas A, Reid G, Edwards J, Lee W (1994) Collagens in the aged human macula. *Graefe's Arch Clin Exp Ophthalmol* 232:133-140
- Marshall J (1987) The ageing retina. physiology or pathology. *Eye* 1:282-295
- Marshall J, Grindle J, Ansell P, Borwien B (1979) Convolution in human rods. *Ophthalmology* 63:181-187
- Martin P, White A, Goodchild A, Wilder H, Sefton A (1997) Evidence that blue-on cells are part of the third geniculocortical pathway in primates. *Eur J Neurosci* 9:1536-1541
- Martinson G (2000) The multifocal electroretinogram in aging and age-related macular degeneration (Thesis): Berkeley: University Of California.
- Martinson G, Haegerstrom-Portnoy G, Verdon W (1999) The multifocal ERG in age-related maculopathy. 1999 Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO) 40:Abstract 3774
- Martinson G, Haegerstrom-Portnoy G, Verdon W (2000) Multifocal ERG abnormalities under soft drusen in age-related maculopathy. 2000 Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO) 42:Abstract 389

- Maruo T, Ikebukuru N, Kawanabe K, Kubuta N (1991) Changes in causes of visual handicaps in Tokyo. *Jpn J Ophthalmol* 35:268-272
- Massay S (1990) Cell types using glutamate as a neurotransmitter in the vertebrate retina. In N.N. Osborne, & G. J. Chader (eds.), *Progress in retinal research*. Oxford, UK: Pergamon Press 9:339-425
- Massof R, Choy D, Sunness J, Johnson M, Rubin G, Fine S (1989) Foveal threshold elevations associated with age-related drusen. *Clin Vis Sci* 4:221-227
- Maturi R, Yu M, Weleber R (2004) Multifocal electroretinographic evaluation of long-term hydroxychloroquine users. *Arch Ophthalmol* 122:973-981
- Mayer M, Spiegler S, Ward B, Glucs A, Kim C (1992a) Foveal flicker sensitivity discriminates ARM risk from healthy eyes. *Invest Ophthalmol Vis Sci* 33:3143-3149
- Mayer M, Spiegler S, Ward B, Glucs A, Kim C (1992b) Mid-frequency loss of foveal flicker sensitivity in early stages of age-related maculopathy. *Invest Ophthalmol Vis Sci* 33:3136-3142
- Mayer M, Ward B, Klein R, Talcott J, Dougherty R, Glucs A (1994) Flicker sensitivity and fundus appearance in pre-exudative age-related maculopathy. *Invest Ophthalmol Vis Sci* 35:1138-1149
- McCarty C, Mukesh B, Guymer R, Baird P, Taylor H (2001a) Cholesterol-lowering medications reduce the risk of age-related maculopathy progression. *Med J Aust* 175:340
- McCarty C, Mukesh B, Mitchell P, Wang J, Taylor H (2001b) The Visual Impairment Project. Risk factors for age-related maculopathy. *Arch Ophthalmol* 119:1455-1462
- Meads C, Hyde C (2003) What is the cost of blindness? *Br J Ophthalmol* 87:1201-1204
- Meigen T, Friedrich A (2002) The reproducibility of multifocal ERG recording [in German]. *Ophthalmologie* 99:713-718
- Merin S, Auerbach E (1970) The central and peripheral retina in macular degenerations. *Arch Ophthalmol* 84:710-718
- Meyers S (1994) A twin study on age-related macular degeneration. *Trans Am Ophthalmol Soc* 92:775-843

- Miceli M, Newsome D, Tate D, Sarphe T (2000) Pathologic changes in the retinal pigment epithelium and Bruch's membrane of fat atherogenic mice. *Curr Eye Res* 20:8-16
- Michels S, Barbarezzo I, Schmidt-Erfurth U (2002) Changes in neovascular membranes and normal choroid blood vessels after multiple photodynamic therapy treatments [in German]. *Ophthalmologie* 99:96-100
- Michels S, Schmidt-Erfurth U (2003) Sequence of early vascular events after photodynamic therapy. *Invest Ophthalmol Vis Sci*. 44:2147-2154
- Midena E, Degli A, Blarzino M, Valenti M, Segato T (1997) Macular function impairment in eyes with early age-related macular degeneration. *Invest Ophthalmol Vis Sci* 38:469-477
- Midena E, Segato T, Blarzino M, Angeli C (1994) Macular drusen and the sensitivity of central visual fields. *Doc Ophthalmol* 88:179-185
- Miller D, Jousseaume A, Holz F (2003) The molecular mechanism of neovascular age-related macular degeneration [in German]. *Ophthalmologie* 100:92-96
- Ming Y, Algvere P, Obergren A, Berglin L, van der Ploeg I, Seregard S, Kvanta A (2003) Subthreshold transpupillary thermotherapy reduces experimental choroidal neovascularisation in the mouse without collateral damage to the neural retina. *Invest Ophthalmol Vis Sci* 45:1969-1974
- Mitchell E, Smith W, Attebo K, Wong J (1995) Prevalence of age-related maculopathy in the Rotterdam Study. *Ophthalmology* 102:1450-1460
- Mitchell P, Smith W, Cumming R, Flood V, Rochtchina E, Wang J (2003) Nutritional factors in the development of age-related eye disease. *Asia Pac J Clin Nutr* 12 (Suppl):5
- Mitchell P, Wang J, Foran S, Smith W (2002a) The five-year incidence of age-related maculopathy lesions: the Blue Mountains Eye Study. *Ophthalmology* 109:1092-1097
- Mitchell P, Wang J, Smith W, Leeder S (2002b) Smoking and the 5-years incidence of age-related maculopathy: The Blue Mountain Eye Study. *Arch Ophthalmol* 120:1357-63
- Miyake Y (1988) Studies of local macular ERG. *Acta Soc Ophthalmol Jpn* 92:1419-1448
- Miyake Y, Shiroyama N, Horiguchi M, Ota I (1989) Asymmetry of focal ERG in human macular region. *Invest Ophthalmol Vis Sci* 30:1743-1749

- Miyake Y, Yanagida K, Kondo M, Yagasaki K, Ohta I (1981) Subjective scotometry and recording of local electroretinogram and visual evoked response system with television monitor of the fovea. *Jap J Ophthalmol* 25:438-448
- Moeller S, Mares J (2003) Ethnic differences in diet and age-related maculopathies. *Int Ophthalmol Clin* 43:47-59
- Mohand-Said S, Hicks D, Leveillard T, Picaud S, Porto F, Sahel J (2001) Rod-cone interactions: developmental and clinical significance. *Prog Retin Eye Res* 20:451-467
- Mohidin N, Yap M, Jacobs R (1999) Influence of age on the multifocal electroretinography. *Ophthalmic Physiol Opt* 19:481-488
- Moisseiev J, Alahalel A, Masuri R, Treister G (1995) The impact of the macular photocoagulation study results on the treatment of exudative age-related macular degeneration. *Arch Ophthalmol* 113:185-189
- Moore D, Hussain A, Marshall J (1995) Age-related variation in the hydraulic conductivity of Bruch's membrane. *Invest Ophthalmol Vis Sci* 36:1290-1297
- Moreland J (1982) Spectral sensitivity measures by motion photometry. *Doc Ophthalmol*:61-66
- Moret F, Doelemeyer A, Schmetterer L, Lambrou G (2004) A novel ophthalmic camera-based fundus-controlled multifocal ERG instrument for monitoring retinal function. 2004 Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO):Abstract 4248
- Moschos M, Apostolopoulos M, Ladas I, Theodossiadis P, Malias J, Moschos M, Theodossiadis G (2001) Assessment of macular function by multifocal electroretinogram before and after epimacular membrane surgery. *Retina* 21:590-595
- Mousa S, Lorelli W, Campochiaro P (1999) Role of hypoxia and extracellular matrix-integrin binding in the modulation of angiogenetic growth factors secretion by retinal pigmented epithelial cells. *J Cell Biochem* 74:135-143
- Mukesh B, Dimitrov P, Leikin S, Wang J, Mitchell P, McCarty C, HR T (2004) Five-year incidence of age-related maculopathy: the Visual Impairment Project. *Ophthalmology* 111:1176-1182
- Mullins R, Aptsiauri N, Hageman G (2000) Structure and composition of drusen associated with glomerulonephritis: implication for the role of complement activation in drusen. *Eye* 15:390-395

- Mullins R, Johnson L, Anderson D, Hageman G (1997) Characterization of drusen-associated glycoconjugates. *Ophthalmology* 104:288-294
- Murata T, He S, Hanga M, Ishibashi T, Xi X, Kim S, Hsueh W, Ryan S, Law R, Hinton D (2000) Peroxisome proliferator-activated receptor-gamma ligands inhibit choroidal neovascularization. *Invest Ophthalmol Vis Sci* 41:2309-2317
- Naarendorp F, Sieving P (1991) The scotopic threshold response of the cat ERG is suppressed selectively by GABA and glycine. *Vision Res* 31:1-15
- Nabeshima T (2001) The effects of aging on the multifocal electroretinogram. *Jpn J Ophthalmol* 45:114-115
- Nabeshima T, Tazawa Y, Mita M, Sano M (2002) Effects of aging on the first and second-order kernels of the multifocal electroretinogram. *Jpn J Ophthalmol* 46:261-269
- Nagasaka K, Horiguchi M, Shimada Y, Yuzawa M (2003) Multifocal electroretinograms in cases of central areolar choroidal dystrophy. *Invest Ophthalmol Vis Sci* 44:1673-1679
- Nagatomo A, Nao-i N, Maruiwa F, Arai M, Sawada A (1998) Multifocal electroretinograms in normal subjects. *Jpn J Ophthalmol* 42:129-135
- Narayanan K, Wadhwa S (1998) Photoreceptor morphogenesis in the human retina: a scanning electron microscopic study. *Anat Rec* 252:133-139
- Nathans J, Thomas D, Hogness D (1986) Molecular genetics of human color vision: the genes encoding blue, green and red pigments. *Science* 232:193-202
- National Research Council Committee on Diet and Health Food and Nutrition Board (1989) Implication for Reducing Chronic Disease Risk. In: *Diet and Health*, Washington DC, National Academy Press:181-189
- Nelson K, Kolb H (2003) ON and OFF pathways in the vertebrate retina and visual system. In: Chalupa, L.M., Werner, J. S. (eds.), *The Visual Neurosciences*. MIT Press, Cambridge:260-278
- Neuringer M, Anderson G, Connor W (1988) The essentially of n-3 fatty acids for the development and function of the retina and brain. *Annu Rev Nutr* 8:517-554
- Newsom R, McAlister J, Saeed M, El-Ghonemy K, McHugh D (2004) Transpupillary thermotherapy (TTT) of classic and occult choroidal neovascularisation in patients with age-related macular degeneration; results at

- 29 months. 2004 Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO):Abstract 5130
- Newsome D, Huh W, Green W (1987) Bruch's membrane age-related changes vary by region. *Curr Eye Res* 6:1211-1221
- Newsome D, Swartz M, Leone N, Elston R, Miller E (1988) Oral zinc in macular degeneration. *Arch Ophthalmol* 106:192-198
- Nguyen Q, Shah S, Van Anden E, Sung J, Vitale S, Campochiaro P (2004) Supplemental oxygen improves diabetic macular edema: a pilot study. *Invest Ophthalmol Vis Sci* 45:617-624
- Nicolas C, Robman L, Tikellis G, Dimitrov P, Dowrick A, Guymer R, McCarty C (2003) Iris colour, ethnic origin and progression of age-related macular degeneration. *Clin Exp Opt* 31:465-469
- Niemeyer G (1969) Elektroretinographie bei Makuladegeneration. *Graefes Arch Clin Exp Ophthalmol* 177:39-51
- Nusinowitz S, Birch DG, Hood D (1994) Focal rod ERG: removing the contribution of scattered light. 1994 Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO) 35:Abstract 1379
- Nusinowitz S, Ridder III W, JR. H (1999) Rod multifocal electroretinogram in mice. *Invest Ophthalmol Vis Sci* 40:2848-2858
- Odel J, Holopigian K, Hood D (1999) Multifocal ERG and threshold perimetry in occult macular dystrophy. *Invest Ophthalmol Vis Sci* 41:513-517
- Ogden T (1973) The oscillatory waves of the primate electroretinogram. *Vision Res* 13:1059-1074
- Oh H, Takagi H, Takagi T, Suzuma K, Otani A, Ishida K, Matsumura M, Ogura Y, Honda Y (1999) The potential angiogenic role of macrophages in the formation of choroidal neovascular membranes. *Invest Ophthalmol Vis Sci* 40:1891-1898
- Ohno-Matsui K, Morita I, Tombran-Tink. J, Mrazek D, Onodera M, Uetama T, Hyano M, Murota S, Mochjizuki M (2001) Novel mechanism for age-related macular degeneration: an equilibrium shift between the angiogenesis factors VEGF and PEDF. *J Cell Physiol* 189:323-333
- Okubo A, Rosa R, Bunce K, Alexander R, Fan J, Bird A, Luthert P (1999) The relationships of age changes in retinal pigment epithelium and Bruch's membrane. *Invest Ophthalmol Vis Sci* 40:443-449

- Olk R, Friberg T, Stickney K, Akduman L, Wong K, Chen M, Levy M, Garcia C, Morse L (1999) Therapeutic benefits of infrared (810-nm) diode laser macular grid photocoagulation in prophylactic treatment of nonexudative age-related macular degeneration: two-year results of a randomized pilot study. *Ophthalmology* 106:2082-2090
- Onozu H, Yamamoto S (2003) Oscillatory potentials of multifocal electroretinogram retinopathy. *Doc Ophthalmol* 106:327-332
- Oosterhuis J, Journee'-de Korver K, Kakebeeke-Kemme H, Bleeker J (1995) Transpupillary thermotherapy in choroidal melanomas. *Arch Ophthalmol* 113:315-321
- Ormerod L, Puklin J, Frank R (1994) Long-term outcomes after the surgical removal of advanced subfoveal neovascular membranes in age-related macular degeneration. *Ophthalmology* 101:1201-1210
- Ostenfeld-Akerblom A (1999) Age-related macular degeneration in Inuit. *Acta Ophthalmol Scand* 77:76-78
- Osterberg G (1935) Topography of the layer of rods and cones in the human retina. *Acta Ophthalmol* 13:1-103
- Otani A, Takagi H, Oh H, Koyama S, Ogura Y, Matumura M, Honda Y (1999) Expressions of angiopoietins and Tie2 in human choroidal neovascular membranes. *Invest Ophthalmol Vis Sci* 40:1912-1920
- Owens S, Bunce C, Brannon A, Wormald R, Bird A, Drusen Laser Study Group (2003) Prophylactic laser treatment appears to promote choroidal neovascularisation in high-risk ARM: results of an interim analysis. *Eye* 17:623-627
- Owsley C, Jackson G, Cideciyan A, Huang Y, Fine S, Ho A, Marguire M, Lolley V, Jacobson S (2000) Psychophysical evidence for rod vulnerability in age-related macular degeneration. *Invest Ophthalmol Vis Sci* 41:267-273
- Owsley C, Jackson G, White M, Edwards D (2001) Delays in rod-mediated dark-adaptation in early age-related maculopathy. *Ophthalmology* 108:1196-1202
- Owsley C, Sloane M, Skalka M, Jackson C (1990) A comparison of the Regan low-contrast letter charts and contrast sensitivity testing in older patients. *Clin Vis Sci* 5:325-334

- Palmowski A, Allgayer R, Heinemann-Vernaleken B (2000) The multifocal ERG in open angle glaucoma- A comparison of high and low contrast recording in high- and low-tension open angle glaucoma. *Doc Ophthalmol* 101:35-49
- Palmowski A, Allgayer R, Heinemann-Vernaleken B, Ruprecht K (2001) First and second order changes in the multifocal electroretinogram of patients with different forms of age related macular degeneration. In *Vision Science and its Application*. OSA Tech Dig Ser, Optical Society of America, Washington, DC::32-35
- Palmowski A, Allgayer R, Heinemann-Vernaleken B, Ruprecht K (2002) Influence of photodynamic therapy in choroidal neovascularization on focal retinal function assessed with the multifocal electroretinogram and perimetry. *Ophthalmology* 109:1788-1792
- Palmowski A, Allgayer R, Heinemann-Vernaleken B, Scherer V, Ruprecht K (2003) Detection of retinal dysfunction in vitelliform macular dystrophy using the multifocal ERG (MF-ERG). *Doc Ophthalmol* 106:145-152
- Palmowski A, Berninger T, Allgayer R, Andrielis H, Heinemann-Vernaleken B, Rudolph G (1999a) The effect of refractive blur on the multifocal electroretinogram. *Doc Ophthalmol* 99:41-54
- Palmowski A, Ruprecht K (2004) Follow up in open angle glaucoma. A comparison of static perimetry and the fast stimulation mfERG. *Doc Ophthalmol* 108:55-60
- Palmowski A, Sutter E, Bearnse M, Fung W (1997) Mapping of retinal function in diabetic retinopathy using the multifocal electroretinogram. *Invest Ophthalmol Vis Sci* 38:2586-2596
- Palmowski A, Sutter E, Bearnse M, Fung W (1999b) Multifocal electroretinogram (MF-ERG) in diagnosis of macular changes. Example: senile macular degeneration [in German]. *Ophthalmologie* 96:166-173
- Panda-Jonas S, Jonas J, Jakobczyk-Zmija M (1995) Retinal photoreceptor density decreases with age. *Ophthalmology* 102:1853-1859
- Park M, Beak S, Ohn Y, Park T (2004) Multifocal electroretinographic findings after intravitreal injection of triamcinolone acetonide on macular edema. 2004 Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO):Abstract 1940

- Parks S, Keating D, Evans A, Williamson T, Jay J, Elliot A (1996-1997) Comparison of the repeatability of the multifocal electroretinogram and Humphrey perimeter. *Doc Ophthalmol* 92:281-289
- Parks S, Keating D, Williamson T, Evans A, Elliott A, Jay J (1996) Functional imaging of the retina using the multifocal electroretinography: a control study. *Br J Ophthalmol* 80:831-834
- Pauleikhoff D (1992) Drusen in Bruch's membrane. Their significance for the pathogenesis and therapy of age-associated macular degeneration. *Ophthalmologie* 89:363-386
- Pauleikhoff D, Barondes M, Minassian D, Chisholm I, Bird A (1991) Drusen as risk factors in age-related macular disease. *Am J Ophthalmol* 109:38-43
- Pauleikhoff D, Chen J, Chisholm I, Bird A (1990a) Choroidal perfusion abnormality with age-related Bruch's membrane change. *Am J Ophthalmol* 109:211-217
- Pauleikhoff D, Harper C, Marshall J, Bird A (1990b) Ageing changes in Bruch's membrane. A histochemical and morphological study. *Ophthalmology* 97:171-178
- Pauleikhoff D, Radermacher M, Spital G, Mueller C, Brumm G, Lommatzsch A, Bird A (2002) Visual prognosis of second eyes in patients with unilateral exudative age-related macular degeneration. *Graefe's Arch Clin Exp Ophthalmol* 240:539-542
- Pauleikhoff D, Spital G, Radermacher M, Brumm G, Lommatzsch A, Bird A (1999) A fluorescein and indocyanine green angiographic study of choriocapillaris in age-related macular disease. *Arch Ophthalmol* 117:1353-1358
- Pauleikhoff D, Zuels S, Sheridah G, Marshall J, Wessing A, Bird A (1992) Correlation between biochemical composition and fluorescein binding of deposits in Bruch's membrane. *Ophthalmology* 99:1548-1553
- Pelli D, Robson J, Wilkins A (1988) The design of a new letter chart for measuring contrast sensitivity. *Clin Vis Sci* 2:187-199
- Penfold P, Gyory J, Hunyor A, Billson F (1995) Exudative macular degeneration and intravitreal triamcinolone. A pilot study. *Aust N Z J Ophthalmol*. 23:293-298
- Penfold PL (1990) Angiogenesis in normal human retinal development: The involvement of astrocytes and macrophages. *Graefe's Arch Clin Exp Ophthalmol* 228:255-263

- Penfold PL (2002) Intravitreal triamcinolone in recurrence of choroidal neovascularisation. *Br J Ophthalmol* 86:600-601
- Penfold PL, Killingsworth M, Sarks S (1985) Senile macular degeneration: The involvement of immunocompetent cells. *Graefe's Arch Clin Exp Ophthalmol* 223:69-76
- Penfold PL, Madigan M, Gillies M, Provis J (2001) Immunological and aetiological aspects of macular degeneration. *Prog Ret Eye Res* 20:385-414
- Penfold PL, Provis J, Furby J, Gatenby P, Billson F (1990) Autoantibodies to retinal astrocytes associated with age-related macular degeneration. *Graefe's Arch Clin Exp Ophthalmol* 228:270-274
- Penn J, Rajaratnam V, Collier R, Clark A (2001) The effect of an angiostatic steroid on neovascularisation in a rat model of retinopathy of prematurity. *Invest Ophthalmol Vis Sci* 42:283-290
- Penrose P, Tzekov R, Sutter E, Fu A, Allen A, Fung W, Oxford K (2002) Multifocal electroretinography evaluation for early detection of retinal dysfunction in patients taking hydroxychloroquine. *Retina* 23:503-512
- Petrukhin K, Koisti M, Bakall B, Li W, Xie G, Marknell T, Sandgren O, Forsman K, Holmgren G, Andreasson S, Vujic M, Bergen A, McGarty-Dugan V, Figueroa D, Austin C, Metzker M, Caskey C, Wadelius C (1998) Identification of the gene responsible for Best macular dystrophy. *Nat Genet* 19:241-247
- Phipps J, Dang T, Vingrys A, Guymer R (2004) Flicker perimetry losses in age-related macular degeneration. *Invest Ophthalmol Vis Sci* 45:3355-3360
- Phipps J, Guymer R, Vingrys A (2003) Loss of cone function in age-related maculopathy. *Invest Ophthalmol Vis Sci* 44:2277-2283
- Phipps J, Guymer R, Vingrys AJ (1999) Temporal sensitivity deficits in patients with high-risk drusen. *Aust Nz J Ophthalmol*. 27:265-267
- Piao C, Kondo M, Nakamura M, Terasaki H, Miyake Y (2003) Multifocal electroretinograms in X-linked retinoschisis. *Invest Ophthalmol Vis Sci* 44:4920-4930
- Piao C, Kondo M, Tanikawa A, Terasaki H, Miyake Y (2000) Multifocal electroretinogram in occult macular dystrophy. *Invest Ophthalmol Vis Sci* 41:513-517

- Piguet B, Wells J, Palmvang I, Wormald R, Chisholm I, Bird A (1993) Age-related Bruch's membrane change: a clinical study of the relative role of heredity and environment. *Br J Ophthalmol* 77:400-403
- Pokorny J, Smith V (1992) Color vision and night vision. In S.J. Ryan, *The Retina*, Mosby, St. Louis:127-142
- Pokorny J, Smith V, Verriest G, Pinkers A, (eds.) (1979) *Congenital and acquired color vision defects*. New York, Grune & Stratton.
- Poloschek C, Rupp V, Krastel H, Holz F (2003) Multifocal ERG recording with simultaneous fundus monitoring using a confocal scanning laser ophthalmoscope. *Eye* 17:159-166
- Poloschek C, Sutter E (2002) The fine structure of multifocal ERG topographies. *J Vision* 2:577-587
- Prenner J, Rosenblatt B, Tolentino M, Gui-Shuang Y, Javornik N, Maguire M, Ho A, The CNVPT Research Group (2003) Risk factors for chorioretinal neovascularisation and vision loss in the fellow eye study of CNVPT. *Retina* 23:307-314
- Pruente C, Schroeder B, Frei J (2001) Multifocal electroretinography after photodynamic therapy in age-related macular degeneration. 2001 Annual Meeting of the Association for Research in Vision and Ophthalmology:Abstract 2377
- Radtke N, Aramant R, Seiler M, Petry H (1999) Preliminary report: indications of improved visual function after retinal sheet transplantation in retinitis pigmentosa patients. *Am J Ophthalmol* 128:384-387
- Rakoczy P, Zhang D, Lay M, Constable I (2000) Accelerated rod outer segment (ROS) derived debris accumulation linked to the production of enzymatically inactive cathepsin D. 2000 Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO):Abstract 2190
- Rakoczy P, Baines M, Kennedy C, Constable I (1996) Correlation between autofluorescent debris accumulation and the presence of partially processed forms of cathepsin D in cultured retinal pigment epithelial cells challenged with rod outer segments. *Exp Eye Res* 63:159-167
- Rangaswamy N, Hood D, Frishman L (2003) Regional variations in local contributions to the primate photopic flash ERG: revealed using the slow-sequence mfERG. *Invest Ophthalmol Vis Sci* 44:3233-3247

- Ranson N, Danis R, Ciulla TA, Pratt LM (2002) Intravitreal triamcinolone in subfoveal recurrence of choroidal neovascularisation after laser treatment in macular degeneration. *Br J Ophthalmol* 86:527-529
- Rapp L, Maple S, Choi J (2000) Lutein and zeaxanthin concentrations in rod outer segment membranes from perifoveal and peripheral human retina. *Invest Ophthalmol Vis Sci*:1200-1209
- Raz D, Seeliger M, Geva A, Percicot C, Lambrou G, Ofri R (2002) The effect of contrast and luminance on mfERG responses in a monkey model of glaucoma. *Invest Ophthalmol Vis Sci* 43:2027-2035
- Rechtman E, Danis R, Pratt L, Harris A (2004) Intravitreal triamcinolone with photodynamic therapy for subfoveal choroidal neovascularisation in age related macular degeneration. *Br J Ophthalmol* 88:344-347
- Reeves G (2004) Update on the immunology, diagnosis and management of systemic lupus erythematosus. *Intern Med J* 34:338-347
- Regan C, De Grip W, Daemen F, Bonting S (1980) Degradation of rhodopsin by a lysosomal fraction of retinal pigment epithelium: biochemical aspects of the visual process. *Exp Eye Res* 30:183-191
- Reichel E, Berrocal A, Ip M, Kroll A, Desai V, Duker J, Puliafito C (1999) Transpupillary thermotherapy of occult subfoveal neovascularisation in patients with age-related degeneration. *Ophthalmology* 106:1908-1914
- Reichel E, Musch D, Mainster M, Blodi B, Ip M (2004) Baseline clinical characteristics of TTT4CNV trial participants. 2004 Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO):Abstract 3132
- Remky A, Lichtenberg K, Arend O (2001) Short wavelength automated perimetry in age-related maculopathy. *Br J Ophthalmol* 85:1432-1436
- Remulla J, Gaudio A, S. M, Sandberg M (1995) Foveal electroretinograms and choroidal perfusion characteristics in fellow eyes of patients with unilateral neovascular age-related macular degeneration. *Br J Ophthalmol* 79:558-561
- Richer S, Stiles W, Statkute L, Pulido J, Frankowski J, Rudy D, Pei K, Tsipursky M, Nyland J (2004) Double-masked, placebo-controlled, randomized trial of lutein and antioxidant supplementation in the intervention of atrophic age-related macular degeneration: the Veterans LAST study (Lutein Antioxidant Supplementation Trial). *Optometry* 75:216-230

- Riva Sanseverino E, Meduri R, Pizzino A, Prantera M, E M (1990) Effects of oxygen-ozone therapy on age-related degenerative retinal maculopathy. *Panminerva Med* 32:77-84
- Rivera A, White K, Stoehr H, Steiner K, Hemmrich N, Grimm T, Jurklies B, Lorenz B, Scholl H, Apfelstedt-Sylla E, Weber B (2000) A comprehensive survey of sequence variation in the ABCA (ABCR) gene in Stargardt disease and age-related macular degeneration. *Am J Hum Gen* 67:800-813
- Robison W, Kuwabara T, Bieri J (1982) The roles of vitamin E and unsaturated fatty acids in the visual process. *Retina* 2:263-281
- Robson A, Moreland J, Pauleikhoff D, Morrissey T, Holder G, Fitzke F, Bird A, van Kuijk F (2003) Macular pigment density and distribution: comparison of fundus autofluorescence with minimum motion photometry. *Vision Res* 43:1765-1775
- Rodieck R, Watanabe M (1993) Survey of the morphology of macaque retinal ganglion cells that project to the pretectum, superior colliculus, and parvicellular laminae of the lateral geniculate nucleus. *J Comp Neurol* 338:289-303
- Rogers A, Danis R, Gao H (2004) Photodynamic therapy with verteporfin combined with intravitreal triamcinolone acetonide for treatment of choroidal neovascularisation. 2004 Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO):Abstract 3157
- Rogers A, Martidis A, PB. G, Puliafito C (2002) Optical coherence tomography findings following photodynamic therapy of choroidal neovascularisation. *Am J Ophthalmol* 134:566-576
- Rohrscheider K, Becker M, Kruse F, Fendrich T, Voelker H (1995) Stability of fixation: results of fundus-controlled examination using scanning laser ophthalmoscope. *Ger J Ophthalmol* 7:197-202
- Rohrscheider K, Bueltmann S, Kiel R, Weimer P, Krastel H, Blankennagel A (2002) Diagnosis of retinal diseases. Comparison between multifocal ERG and fundus perimetry-a case study [in German]. *Ophthalmologe* 99:695-702
- Rohrschneider K, Bueltmann S (2001) Fundus-controlled functional evaluation in macular diseases with the scanning laser ophthalmoscope [in German]. *Ophthalmologe*:3-9

- Rosenfeld P, Verteporfin in minimally (VIM) Study Group (2004) Verteporfin in minimally classic CNV due to AMD (VIM). 2004 Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO):Abstract 2273
- Rosenfeld T (1987a) Prevalence and causes of blindness in Greenland. *Arct Med Res* 46:13-17
- Rosenfeld T (1987b) Prevalence of blindness caused by senile macular degeneration in Greenland. *Arct Med Res* 46:64-70
- Roth D, Yarian S, Gree S, Leff S, Friedmann E, Keyser B, Wheatly H, Modi A (2004) Intravitreal triamcinolone combined with photodynamic therapy for choroidal neovascularisation associated with age-related macular degeneration. 2004 Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO):Abstract 2218
- Rożanowska M, Jarvis-Evans J, Korytowski W, Boulton M, Burke J, Sarna T (1995) Blue light-induced reactivity of retinal age pigment. In vitro generation of oxygen-reactive species. *J Biol Chem* 270:18825-18830
- Rubin G, Bressler N, and the Treatment of Age-Related Macular Degeneration with Photodynamic Therapy (TAP) Study Group (2002) Effects of Verteporfin therapy on contrast sensitivity. Results from the Treatment of Age-Related Macular Degeneration With Photodynamic Therapy (TAP) investigation-TAP report No.4. *Retina* 22:536-544
- Rudolph G, Kalpadakis P, Bechmann M, La Rocca G, Hormann C, Berninger T (2002) Scanning laser ophthalmoscope-evoked multifocal-ERG (SLO-m-ERG) by using short m-sequences. *Eur J Ophthalmol* 112:109-116
- Ruiz-Moreno J, de la Vega C, Zarbin M (2003) Macular atrophy after photocoagulation of soft drusen. *Retina* 23:315-321
- Rüther K, Breidenback K, Schwartz R, Hassenstein A, Richard G (2003) Testing central retinal function with multifocal electroretinography before and after photodynamic therapy. [in German]. *Ophthalmologe* 100:459-464
- Ryan S, Hinton D, Murata T (2001) Choroidal neovascularisation. In: Ryan SJ, ed *Retina*. 3rd. St. Louis: Mosby:1005-1006
- Saito T (1987) Physiological and morphological differences between On- and Off-center bipolar cells in the vertebrate retina. *Vision Res* 27:135-142

- Sakmar T (1998) Rhodopsin: a prototypical G protein-coupled receptor. *Prog Nucleic Acid Res Mol Biol* 59:1-34
- Salinas-Alaman A, Garcia-Layana A, Moreno-Montanes J (2003) Overtreatment of transpupillary thermotherapy for choroidal neovascularisation. *Acta Ophthalmol Scand* 81:197-198
- Sandberg M, Ariel M (1977) A hand-held two channel stimulator ophthalmoscope. *Arch Ophthalmol* 95:1881-1882
- Sandberg M, Berson E (1977) Blue and green cone mechanism on the spectral sensitivity of the fovea. *Mod Probl Ophthalmol* 11:219-223
- Sandberg M, Gaudio A (1995) Slow photostress recovery and disease severity in age-related macular degeneration. *Retina* 15:407-412
- Sandberg M, Miller S, Gaudio A (1993) Foveal cone ERGs in fellow eyes of patients with unilateral neovascular age-related macular degeneration. *Invest Ophthalmol Vis Sci* 34:3477-3480
- Sandberg M, Pawlyk B, Berson E (1996) Isolation of focal rod electroretinograms from the dark-adapted human eye. *Invest Ophthalmol Vis Sci* 37:930-934
- Sankeralli M, Chen J, Metha A, Mullen K (2000) Evidence for mild blue-yellow colour vision deficits immediately following fluorescein angiography. *Invest Ophthalmol Vis Sci* 41:137-141
- Sarks J, Sarks S, Killingworth M (1988) Evolution of geographic atrophy of the retinal pigment epithelium. *Eye* 2:552-577
- Sarks J, Sarks S, Killingworth M (1994) Evolution of soft drusen in age-related macular degeneration. *Eye* 8:269-283
- Sarks S (1976) Aging and degeneration in the macular region: a clinico-pathological study. *Br J Ophthalmol* 60:324-341
- Sarks S (1980) Council Lecture. Drusen and their relationship to senile macular degeneration. *Aust J Ophthalmol*. 8:117-130
- Sarks S, Arnold J, Sarks J (1996) Prophylactic perifoveal laser treatment of soft drusen. *Aust NZ J Ophthalmol*. 24:15-26
- Sarks S, Killingsworth M, Sarks J (1999) Early drusen formation in the normal and aging eye and their relation to age related maculopathy: a clinicopathological study. *Br J Ophthalmol* 83:358-368
- Sasseville A, Fredette M, Chakor H, Lachapelle P, Grondin V, Tardif Y, Lalonde G, Cinq-Mars B, MacDonald I, Hebert M (2004) Reproducibility of multifocal

- ERG (mfERG) in patients with macular disease. 2004 Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO):Abstract 4234
- Schachat A, Hyman L, Leske M, Connell A, Wu S (1995) Features of age-related macular degeneration in the black African. *Br J Ophthalmol* 113:728-735
- Schefrin B, Werner J, Plach M, Tutlout N, Switkes E (1992) Sites of age-related sensitivity loss in a short-wave cone pathway. *J Opt Soc Am A* 9:355-363
- Schlingemann R (2004) Role of growth factors and the wound healing response in age-related macular degeneration. *Graefe's Arch Clin Exp Ophthalmol* 242:91-101
- Schloetzer-Schrehardt U, Viestenz A, Naumann G, Laqua H, Michels S, Schmidt-Erfurth U (2002) Dose-related structural effects of photodynamic therapy on choroidal and retinal structures of human eyes. *Graefe's Arch Clin Exp Ophthalmol* 240:748-757
- Schmidt J, Rodrigues E, Meyer C, Kroll P (2003) Is membrane extraction in cases of exudative age-related macular degeneration still up-to-date? A 4-year resume. *Doc Ophthalmol* 217:401-417
- Schmidt S, Peisch R (1985) Melanin concentration in normal human retinal pigment epithelium. *Invest Ophthalmol Vis Sci* 27:1063-1067
- Schmidt-Erfurth U (1999) Indocyanine green angiography and retinal sensitivity after photodynamic therapy of subfoveal choroidal neovascularisation. *Semin Ophthalmol* 14:35-44
- Schmidt-Erfurth U (2001) Recommendations for indication and treatment [in German]. *Ophthalmologie* 98:216-230
- Schmidt-Erfurth U, Elsner H, Terai N, Benecke A, Dahmen G, Michels S (2004) Effects of Verteporfin therapy on central visual field function. *Ophthalmology* 111:931-939
- Schmidt-Erfurth U, Hasan A (2000) Mechanism of action of photodynamic therapy with verteporfin for the treatment of age-related macular degeneration. *Surv Ophthalmol* 45:195-214
- Schmidt-Erfurth U, Hasan A, Gragoudas E, Michaud N, Flotte T, Birngruber R (1994) Vascular targeting in photodynamic occlusion of subretinal vessels. *Ophthalmology* 101:1953-1961

- Schmidt-Erfurth U, Laqua H, Schlotzer-Schrehard U, Viestenz A, Naumann G (2002a) Histopathological changes following photodynamic therapy in human eyes. *Arch Ophthalmol* 120:835-844.
- Schmidt-Erfurth U, Michels S (2003) Changes in confocal indocyanine green angiography through two years after photodynamic therapy with verteporfin. *Ophthalmology* 110:1306-1314
- Schmidt-Erfurth U, Michels S, Barbazetto I, Laqua H (2002b) Photodynamic effects on choroidal neovascularisation and physiological choroid. *Invest Ophthalmol Vis Sci* 43:830-841
- Schmidt-Erfurth U, Schlotzer-Schrehard U, Cursiefen C, Michels S, Beckendorf A, Naumann G (2003) Influence of photodynamic therapy on expression of vascular endothelial growth factor (VEGF), VEGF receptor 3, and pigment epithelium-derived factor. *Invest Ophthalmol Vis Sci* 44:4473-4480
- Schneider U, Kuck H, Inhoffen W, Kreissig I (1993) Fundus-controlled microperimetry with the scanning laser ophthalmoscope in macular diseases [in German]. *Klin Monatsbl Augenheilkd* 203:212-218
- Schneider U, Kuck H, Inhoffen W, Kreissig I (1996) Fundus oriented microperimetry with the scanning laser ophthalmoscope in age-induced macular degeneration, [in German]. *Klin Monatsbl Augenheilkd* 209:8-13
- Scholl H, Bellmann C, Dandekar S, Bird A, Fitzke F (2004) Photopic and scotopic fine matrix mapping of retinal areas of increased fundus autofluorescence in patients with age-related maculopathy. *Invest Ophthalmol Vis Sci* 45:574-583
- Scholl H, Peto T, Dandekar S, Bunce C, Xing W, Jenkins S, Bird A (2003) Inter- and intra-observer variability in grading lesions of age-related maculopathy and macular degeneration. *Graefe's Arch Clin Exp Ophthalmol* 214:39-47
- Scholl H, Schuster A, Vontheim R, Zrenner E (2002) Mapping of retinal function in Best macular dystrophy using multifocal electroretinography. *Vision Res* 42:1053-1061
- Schuett F, Davis S, Koptiz J, Holz F, Boulton M (2000) Photodamage to human RPE cells by A2-E, a retinoid component of lipofuscin. *Invest Ophthalmol Vis Sci* 41:2303-2308
- Seddon J, Ajani U, Sperduto R, Hiller R, Blair N, Burton T, Farber M, Gragoudas E, Haller J, Miller D, et al (1994) Dietary carotenoids, vitamins A, C, and E, and

- advanced age-related macular degeneration. Eye Disease Case-Control Study Group. *Jama* 272:1413-1420
- Seddon J, Cote J, Rosner B (2003) Progression of age-related macular degeneration: association with dietary fat, transunsaturated fat, nuts and fish intake. *Arch Ophthalmol* 121:1728-1737
- Seddon J, Rosner B, Sperduto R, Yannuzzi L, Haller J, Blair N, Willett W (2001) Dietary fat and risk for advanced age-related macular degeneration. *Arch Ophthalmol* 401-404:1191-1199
- Seeliger M, Jurklies B, Kellner U, Palmowski A, Bach M, Kretschmann U (2001a) Multifocal electroretinography (mfERG) [in German]. *Ophthalmologe* 98:1112-1129
- Seeliger M, Kretschmann U, Apfelstedt-Sylla E, Ruether K, Zrenner E (1998a) Multifocal electroretinography in retinitis pigmentosa. *Am J Ophthalmol* 28:214-226
- Seeliger M, Kretschmann U, Apfelstedt-Sylla E, Zrenner E (1998b) Implicit time topography of multifocal electroretinograms. *Invest Ophthalmol Vis Sci* 39:718-723
- Seeliger M, Zrenner E, Apfelstedt-Sylla E, Jaissle G (2001b) Identification of Usher syndrome subtypes by ERG implicit time. *Invest Ophthalmol Vis Sci* 42
- Seiple W, Greenstein V, Holopigian K, Carr R, Hood D (2002) A method for comparing psychophysical and multifocal electroretinographic increment thresholds. *Vision Res* 42:257-269
- Seiple W, Siegel I, Carr R, Mayron C (1986a) Evaluating macular function using the focal ERG. *Invest Ophthalmol Vis Sci* 27:1123-1130
- Seiple W, Siegel I, Carr R, Mayron C (1986b) Objective assessment of modulation transfer functions using the focal ERG. *Am J Ophthalmol* 63:1-6
- Seiple W, Vajaranant T, Szlyk J, Clemens C, Holopigian K, Paliga J, Badawi D, Carr R (2003) Multifocal electroretinography as a function of age: the importance of normative values for older adults. *Invest Ophthalmol Vis Sci* 44:1783-1792
- Selgrade M, Repacholi M, Koren H (1997) Ultraviolet radiation-induced immune modulation: potential consequences for infectious, allergic, and autoimmune disease. *Environ Health Perspect* 105:332-334

- Shamsi F, Boulton M (2001) Inhibition of RPE lysosomal and antioxidants activity by age pigment lipofuscin. *Invest Ophthalmol Vis Sci* 42:3041-3046
- Sharpe L, Stockman A (1999) Rod pathways: the importance of seeing nothing. *Trends Neurosci* 22:497-504
- Shibuki H, Katai N, Kuroiwa S, Kurokawa T, Arai J, Matsumoto K, Nakamura T, Yoshimura N (2002) Expression and neuroprotective effect of hepatocyte growth factor in retinal ischemia-reperfusion injury. *Invest Ophthalmol Vis Sci* 43:528-536
- Shikano S, Shimuzu K (1968) Atlas of fluorescence fundus angiography. Philadelphia: W.B. Saunders Company.
- Shimada Y, Horiguchi M (2003) Stray light-induced multifocal electroretinograms. *Invest Ophthalmol Vis Sci* 44:1245-1251
- Shimada Y, Li Y, Barse M, Sutter E, Fung W (2001) Assessment of early retinal changes in diabetes using a new multifocal ERG protocol. *Br J Ophthalmol* 85:414-419
- Sickel W (1972) Oxidative stress: from basic research to clinical application. In Fourtes MGF (ed.), *Handbook of Sensory Physiology*. Berlin, Springer-Verlag:667-727
- Sieving P, Murayama K, Naarendorp F (1994) Push-pull model of the primate photopic electroretinogram: a role for hyperpolarisation neurons shaping the b-wave. *Vis Neurosci* 11:519-532
- Sigel H (1983) Metal ions in biological systems. In: Zinc and its role in biology and nutrition. Dekker, New York.
- Sigelman J (1991) Foveal drusen resorption one year after perifoveal laser photocoagulation. *Ophthalmology* 98:1379-1383
- Silvestri T, Johnson P, Hughes A (1994) Is genetic predisposition an important risk factor in age-related macular disease? *Eye* 8:564-568
- Singerman L, Verteporfin with Altered (delayed) Light In Occult (VALIO) Study Group (2004) Verteporfin with altered (delayed) light in occult (VALIO). 2004 Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO):Abstract 2274
- Sjostrand J (1979) Contrast sensitivity in macular disease using a small-field and large-field TV system. *Acta Ophthalmol* 1983:832-846

- Slaughter N, Miller R (1983) An excitatory amino acid antagonist blocks cone input to sign-conserving second-order retinal neurons. *Science* 211:182-185
- Smiddy W, Fine S (1984) Prognosis of patients with bilateral macular drusen. *Ophthalmology* 91:271-277
- Smith D, Assink J, Klein R, Mitchell P, Klaver C, BE. K, Hofman A, Jensen S, Wang J, de Jong P (2001) Risk factors for age-related macular degeneration: Pooled findings from three continents. *Ophthalmology* 108:697-701
- Smith D, Fenn P, Drummond M (2004) Cost effectiveness of photodynamic therapy with verteporfin for age related macular degeneration: the UK case. *Br J Ophthalmol* 85:1107-1112
- Smith V, Ernest T, Pokorny J (1976) Effect of hypoxia on FM 100-hue test performance. *Mod Probl Ophthalmol* 17:248-256
- Smith V, Pokorny J, Diddie K (1988) Color matching and Stiles-Crawford effect in observers with early age-related macular degeneration. *J Opt Soc Am A* 5:2113-2121
- Smith W, Mitchell E, Leeder S, Wang J (1998) Plasma fibrinogen levels, other cardiovascular risk factors, and age-related maculopathy. *Arch Ophthalmol* 116:583-587
- Smith W, Mitchell P, Leeder S (2000) Dietary fat and fish intake and age-related maculopathy. *Arch Ophthalmol* 118:401-404
- Smith W, Mitchell P, Wang J (1997) Gender, oestrogen, hormone replacement and age-related macular degeneration: results from the Blue Mountains Eye Study. *Aust NZ J Ophthalmol* 25:13-15
- Smith W, Mitchell W, Leeder S (1996) Smoking and age-related maculopathy: the Blue Mountain Eye Study. *Arch Ophthalmol* 114:1518-1523
- Snodderly D, Auran J, Delori F (1984) The macular pigment. II. Spatial distribution in primate retinas. *Invest Ophthalmol Vis Sci* 25:674-685
- Snodderly D, Mares J, Wooten B, Oxton L, Gruber M, Ficek T, for the CAREDS Macular Pigment Study Group (2004) Macular pigment measurement by heterochromatic photometry in older subjects: The Carotenoids and Age-Related Eye Disease Study. *Invest Ophthalmol Vis Sci* 45:531-538
- Snow K, Cote J, Weining Y, Davis N, Seddon J (2002) Association between reproductive and hormonal factors and age-related maculopathy in postmenopausal women. *Am J Ophthalmol* 130:842-848

- Snow K, Seddon J (1999) Do age-related macular degeneration and cardiovascular diseases share common antecedents? *Ophthalm Epidemiol* 6:125-143
- Solomons N, Russell R (1980) The interaction of vitamin A and zinc: implications for human nutrition. *Am J Clin Nut* 33:2031-2040
- Soubrane G, Francais C, Koenig F (1990) Occult subretinal new vessels in age-related macular degeneration: natural history and early laser treatment. *Ophthalmology* 97:649-657
- Spaide R (2003) Fundus autofluorescence and age-related macular degeneration. *Ophthalmology* 110:392-399
- Spaide R (2004) Combined photodynamic therapy with verteporfin and intravitreal triamcinolone for juxtafoveal choroidal neovascularisation. 2004 Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO):Abstract 2219
- Spaide R, Armstrong D, Browne R (2003a) Choroidal neovascularisation in age-related macular degeneration-what is the cause? *Retina* 23:595-614
- Spaide R, Ho-Spaide W, Browne R, Armstrong D (1999) Characterization of lipid peroxides in Bruch's membrane. *Retina* 19:141-147
- Spaide R, Sorenson J, Maranan L (2003b) Combined photodynamic therapy with verteporfin and intravitreal triamcinolone acetate for choroidal neovascularization. *Ophthalmology* 110:1517-1525
- Spraul C, Lang G, Grossniklaus H, Lang G (1999) Histopathologic and morphometric analysis of the choroid, Bruch's membrane, and the retinal pigment epithelium in postmortem eyes with age-related macular degeneration and histologic examination of surgically excised choroidal neovascular membranes. *Surv Ophthalmol* 44:10-32
- Starita C, Hussain A, Marshall J (1995) Decreasing hydraulic conductivity of Bruch's membrane: relevance to photoreceptor survival and lipofuscinoses. *Am J Med Genet* 57:235-237
- Steinmetz R, Haimovici R, Jubb C, Fitzke F, Bird A (1993) Symptomatic abnormalities of dark adaptation in patients with age-related Bruch's membrane change. *Br J Ophthalmol* 77:549-554
- Stockman A, Sharpe L (1998) Human cone spectral sensitivities: A progress report. *Vision Res* 32:433-446

- Stockman A, Sharpe L (2000) The spectral sensitivities for the middle- and long-wavelength sensitive cones derived from measurements in observers of known genotype. *Vision Res* 40:1711-1737
- Strahlman E, Fine S, Hillis A (1983) The second eye of patients with senile macular degeneration. *Arch Ophthalmol* 101:1191-1193
- Stur M, Tittl M, Reitner A, Meisinger V (1996) Oral zinc and the second eye in age-related macular degeneration. *Invest Ophthalmol Vis Sci* 37:1225-1235
- Stur M, Verteporfin Early Retreatment (VER) Study Group (2004) Verteporfin early retreatment (VER)-12 months results of a phase IIIb controlled clinical trial. 2004 Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO):2275
- Submacular Surgery Trials Pilot Study Investigators (2000a) Submacular surgery trials randomized pilot trial of laser photocoagulation versus surgery for recurrent choroidal neovascularization secondary to age-related macular degeneration: I. Ophthalmic outcomes submacular surgery trials pilot study report number 1. *Am J Ophthalmol* 130:387-407
- Submacular Surgery Trials Pilot Study Investigators (2000b) Submacular surgery trials randomized pilot trial of laser photocoagulation versus surgery for recurrent choroidal neovascularization secondary to age-related macular degeneration: II. Quality of life outcomes submacular surgery trials pilot study report number 2. *Am J Ophthalmol* 130:408-418
- Sunness J, Johnson M, Massof R, Marcus S (1988) Retinal sensitivity over drusen and nondrusen areas. *Arch Ophthalmol* 106:1081-1084
- Sunness J, Massof R (1986) Focal electro-oculogram in age-related macular degeneration. *Am J Optom Physiol Op* 63:7-11
- Sunness J, Massof R, Bressler N, Bressler S (1989a) S-cone pathway sensitivities in eyes with high risk and low risk drusen characteristics. *Appl Opt* 28:1158-1164
- Sunness J, Massof R, Johnson M, Bressler N, Bressler S, Fine S (1989b) Diminished foveal sensitivity may predict the development of advanced age-related macular degeneration. *Ophthalmology* 96:375-381
- Sunness J, Massof R, Johnson M, Finkelstein D, Fine S (1985) Peripheral retinal function in age-related maculopathy. *Arch Ophthalmol* 103:811-816

- Sunness J, Rubin R, Applegate C, Bressler N, Marsh M, Hawkins B, Haselwood D (1997) Visual function abnormalities and prognosis in eyes with age-related geographic atrophy of the macula and good visual acuity. *Ophthalmology* 104:1677-1691
- Suter M, Reme C, Grimm C, Wenzel A, Jaattela M, Esser P, Kociok N, Leist M, Richter C (2000) Age-related macular degeneration. The lipofuscin component N-retinyl-N-retinylidene ethanolamine detaches proapoptotic proteins from mitochondria and induces apoptosis in mammalian retinal pigment epithelial cells. *J Biol Chem* 15:39625-39630
- Sutter E (2000) The interpretation of multifocal binary kernels. *Doc Ophthalmol* 100:49-75
- Sutter E (2001) Imaging visual function with the multifocal m-sequence technique. *Vision Res* 41:1241-1255
- Sutter E, Bearnse M (1995) Extraction of a ganglion cell component from the corneal response. In *Vision science and its Application*, OSA Tech Dig Ser, Optical Society of America, Washington, DC 1:310-313
- Sutter E, Bearnse M (1998) The retinal topography of local and lateral gain control mechanisms. In: *Vision Science and its Applications*. OSA Tech Dig Ser, Optical Society of America, Washington, DC 1:20-23
- Sutter E, Bearnse M (1999) The optic nerve head component of the human ERG. *Vis Res* 39:419-436
- Sutter E, Shimada Y, Li Y, Bearnse MJ (1999) Mapping inner retinal function through enhancement of adaptative components in the M-ERG. In *Vision Science and its Application*. OSA Tech Dig Ser, Optical Society of America, Washington, DC:52-55
- Sutter E, Tran D (1992) The field topography of ERG components in man-I. the photopic luminance response. *Vision Res* 32:433-446
- Suzuki S, Horiguchi M, Tanikawa A, Miyake Y, Kondo M (1998) Effect of age on short-wavelength sensitive cone electroretinogram and long- and middle-wavelength sensitive cone electroretinogram. *Jap J Ophthalmol* 42:424-430
- Swann P, Lovie-Kitchin J (1991) Age-related maculopathy. II: the nature of the central visual field loss. *Ophthal Physiol Opt* 11:59-70
- Tam W, Chan H, Brown B, Yap M (2004) Effects of different degrees of cataract on the multifocal electroretinogram. *Eye* 18:691-696

- Tamai K, Spaide R, Ellis E, Iwabuchi S, Ogura Y, Armstrong D (2002) Lipid hydroperoxide stimulates subretinal choroidal neovascularisation in the rabbit. *Exp Eye Res* 74:301-308
- Taylor H, Tikellis G, Robman L, McCarty C, McNeil J (2002) Vitamin E supplementation and macular degeneration: randomized controlled trial. *Br Med J* 325:11
- Teichmann K (1997) Treatment of macular degeneration, according to Bangerter. *Eur Med Res* 2:445-454
- Teikari J, Laatikainen L, Virtamo J, Haukka J, Rautalahti M, Liesto K, Albanes D, Taylor P, Heinonen O (1998) Six-year supplementation with alpha-tocopherol and beta-carotene and age-related macular degeneration. *Acta Ophthalmol Scand* 76:224-229
- Terasaki H, Ishikawa K, Niwa Y, Piao C-H, Niwa T, Kondo M, Ito Y, Miyake Y (2004) Changes in focal macular ERGs after macular translocation surgery with 360 degrees retinotomy. *Invest. Ophthalmol. Vis. Sci.* 45:567-573
- The Alpha-Tocopherol Beta Carotene Cancer Prevention Study Group (1994) The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group. *N Engl J Med.* 330:1029-1035
- The Anecortave Acetate Clinical Study Group (2003) Anecortave acetate as monotherapy for the treatment of subfoveal lesions in patients with exudative age-related macular degeneration (AMD): interim (month 6) analysis of clinical safety and efficacy. *Retina* 23:14-23
- The Beaver Dam Eye Study (2002) Ten-year incidence and progression of age-related maculopathy. *Ophthalmology* 109:1776-1779
- The Choroidal Neovascularisation Prevention Trial Research Group (1998) Choroidal neovascularisation in the choroidal neovascularisation prevention trial. *Ophthalmology* 105:1364-1372
- The Eye Disease Case-Control Study Group (1992) Risk factors for neovascular age-related macular degeneration. *Arch Ophthalmol* 110:1701-1708
- Theodossiadis G, Theodossiadis P, Malias J, Moschos M, Moschos M (2002) Preoperative and postoperative assessment by multifocal electroretinography in the management of optic disc pits with serous macular detachment. *Ophthalmology* 109:2295-2302

- Thomas D, Duguid G (2004) Optical coherence tomography-a review of the principles and contemporary uses in retinal investigation. *Eye* 18:561-570
- Thomas J (2001) Retinal pigment epithelial tear after transpupillary thermotherapy for choroidal neovascularisation. *Am J Ophthalmol* 131:662-664
- Thomas J, Bachowski G, Girotti A (1986) Inhibition of cell membrane lipid peroxidation by cadmium- and zinc- metallothioneins. *Biochem Biophys Acta* 884:448-461
- Thomas M, Dickinson J, Melberg N, Ibanez H, Dhaliwal R (1994) Visual results after surgical removal of subfoveal choroidal neovascular membranes. *Ophthalmology* 101:1384-1396
- Thomson L, Toyoda Y, Delori F, Garnett K, Wong Z-Y, Nichols C, Cheng K, Craft N, Dorey C (2002) Long term dietary supplementation with zeaxanthin reduces photoreceptor death in light-damaged Japanese quail. *Exp Eye Res* 75:529-542
- Tolentino M, Miller S, Gaudio A, Sandberg M (1994) Visual field deficits in early age-related macular degeneration. *Vis Res* 34:409-413
- Tomany S, Cruickshanks K, Klein R, Klein B, Knudtson M (2004a) Sunlight and the 10 year incidence of age-related maculopathy. *Arch Ophthalmol* 122:750-757
- Tomany S, Wang J, van Leeuwen R, et al (2004b) Risk factors for incident age-related macular degeneration: Pooled findings from three continents. *Ophthalmology* 111:1280-1287
- Treatment of Age-Related Macular Degeneration With Photodynamic Therapy (TAP) and Verteporfin Therapy (VIP) Study Groups (2003) Photodynamic therapy of subfoveal choroidal neovascularisation with verteporfin. Fluorescein angiographic guidelines for evaluation and treatment--TAP and VIP report No. 2. *Arch Ophthalmol* 121:1253-1268
- Treatment of Age-Related Macular Degeneration With Photodynamic Therapy (TAP) Group (1999) Photodynamic therapy of subfoveal choroidal neovascularisation in age-related macular degeneration with verteporfin. *Arch Ophthalmol* 117:1329-1345
- Treatment of Age-Related Macular Degeneration With Photodynamic Therapy (TAP) Study Group (2001) Photodynamic therapy of subfoveal choroidal neovascularisation in age-related macular degeneration with verteporfin: two-

- year results of 2 randomized clinical trials-TAP report 2. *Arch Ophthalmol* 119:198-207
- Treatment of Age-Related Macular Degeneration With Photodynamic Therapy (TAP) Study Group (2002) Verteporfin therapy for subfoveal choroidal neovascularisation in age-related macular degeneration: three-year results of an open-label extension of 2 randomized clinical trials --TAP report no. 5. *Arch Ophthalmol* 120:1307-1314
- Trieschmann M, Spital G, Lommatzsch A, van Kuijk E, Fitzke F, Bird A, Pauleikhoff D (2003) Macular pigment: quantitative analysis on autofluorescence images. *Graefe's Arch Clin Exp Ophthalmol* 241:1006-1012
- Tucker C, Chen L, Judkins M, Farmer J, Gill S, Drolet D (1999) Detection and plasma pharmacokinetics of an anti-vascular endothelial growth factor oligonucleotide-aptamer (NX1838) in rhesus monkeys. *J Chromatogr B Biomrd Sci Appl* 732:203-212
- Tufail A, Neveu M, Dowler J, Holder G (2004) Longitudinal evaluation of the multifocal electroretinogram (mfERG) for determining outcome of PDT for choroidal neovascularisation. 2004 Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO):Abstract 3177
- Tuo J, Bojanowski C, Chan C (2004) Genetic factors of age-related macular degeneration. *Prog Ret Eye Res* 23:229-249
- Twig G, Levy H, Perlman I (2003) Color opponency in horizontal cells of the vertebrate retina. *Prog Ret Eye Res* 22:31-68
- Tzekov R, Gerth C, Werne J (2004) Senescence of human multifocal electroretinogram components: a localized approach. *Graefe's Arch Clin Exp Ophthalmol* 242:549-560
- Ulbig M, Kampik A (1993) Stage-related therapy of diabetic maculopathy. *Ophthalmologie* 90:395-414
- Usui S, Nagasaka E (1994) Spatial distribution of local flash electroretinogram by multi-input stimulation. *Doc Ophthalmol* 88:57-63
- Vaicaitiene R, Luksiene D, Paunksnis A, Cerniauskiene L, Domarkiene S, Cimbalas A (2003) Age-related maculopathy and consumption of fresh vegetables and fruits in urban elderly. *Medicina (Kaunas)* 39:1231-1236

- van der Schaft T, de Bruijn W, Mooy C, Ketelaars D, de Jong P (1991) Is basal laminar deposit unique for age-related macular degeneration? *Arch Ophthalmol* 109:420-425
- van der Schaft T, Mooy C, de Bruijn W, Bosman F, de Jong P (1994) Immunohistochemical light and electron microscopy of basal laminar deposit. *Graefe's Arch Clin Exp Ophthalmol* 232:40-46
- van Leeuwen R, Ikram M, Vingerling J, Witteman J, Hofman A, de Jong P (2003a) Blood pressure, atherosclerosis and the incidence of age-related maculopathy: the Rotterdam Study. *Invest Ophthalmol Vis Sci* 44:3771-3777
- van Leeuwen R, Klaver C, Vingerling J, Hofman A, de Jong P (2003b) The risk and natural course of age-related maculopathy. Follow-up at 6 1/2 years in the Rotterdam Study. *Arch Ophthalmol* 121:519-526
- van Leeuwen R, Klaver C, Vingerling J, Hofman A, van Duijn C, Stricker B, de Jong P (2004a) Cholesterol and age-related macular degeneration: is there a link? *Am J Ophthalmol* 137:750-752
- van Leeuwen R, Tomany S, Wang F, Klein R, Mitchell E, Hofman A, Klein B, Vingerling J, Cumming R, de Jong P (2004b) Is medication use associated with the incidence of early age-related maculopathy. *Ophthalmology* 111:1169-1175
- van Leeuwen R, Vingerling J, Hofman A, de Jong P, Stricker B (2003c) Cholesterol lowering drugs and risk of age related maculopathy: prospective cohort study with cumulative exposure measurement. *BMJ* 326:255-256
- VandenLangenberg G, Mares-Perlman J, Klein R, Klein B, Brady W, Palta M (1998) Associations between antioxidant and zinc intake and the 5-year incidence of early age-related maculopathy in the Beaver Dam Eye Study. *148:204-214*
- Vandewoude M, Vandewoude M (1987) Vitamin E status in normal population: the influence of age. *J Am Coll Nutr* 6:307-311
- VanNewkirk M, Nanjan M, Wang J, Mitchell P, Taylor H, McCarty C (2000) The prevalence of age-related maculopathy: the visual impairment project. *Ophthalmology* 107:1593-1600
- Vardi N, Zhang L, Payne J, Sterling P (2000) Evidence that different cation chloride cotransporters in retinal neurons opposite response to GABA. *J Neurosci* 20:7657-7663

- Varner D, Cook J, Schneck M, McDonald M, Teller D (1985) Tritan discriminations by 1- and 2-month-old human infants. *Vision Res* 25:821-831
- Vaughan C, Murphy M, Buckley B (1996) Statins do more than just lower cholesterol. *Lancet* 348:1079-1082
- Verdon W, Haegerstrom-Portnoy G (1998) Topography of the multifocal electroretinogram. *Doc Ophthalmol* 95:73-90
- Verteporfin In Photodynamic Therapy (VIP) Study Group (2001a) Photodynamic therapy of subfoveal choroidal neovascularisation in pathologic myopia. 1-year result of a randomized clinical trial--VIP report no. 1. *Ophthalmology* 108:841-852
- Verteporfin In Photodynamic Therapy (VIP) Study Group (2001b) Verteporfin therapy of subfoveal choroidal neovascularisation in age-related macular degeneration: two-year results of a randomized clinical trial including lesions with occult with no classic choroidal neovascularisation--verteporfin in photodynamic therapy report 2. *Am J Ophthalmol* 131:541-560
- Vingerling J, Dielemans I, Hofman A, Groebbe D, Hijmering M, Kramer C, de Jong P (1995) The prevalence of age-related maculopathy in the Rotterdam Study. *Ophthalmology* 102:205-210
- Vingrys A, Atchison D, Bowman K (1992) The use of colour differences vectors in diagnosing congenital vision deficiencies with the Farnsworth-Munsell 100-hue test. *Ophthal Physiol Opt* 12:38-45
- Vingrys A, King-Smith P (1988) A quantitative scoring technique for panel tests of color vision. *Invest Ophthalmol Vis Sci* 29:50-63
- von Rueckmann A, Fitzke F, Bird A (1995) Distribution of fundus autofluorescence with a scanning laser ophthalmoscope. *Br J Ophthalmol* 79:407-412
- Wachtlin J, Behme T, Heiman H, Kellner U, Foester M (2003) Concentric retinal pigment epithelium atrophy after single photodynamic therapy. *Graefe's Arch Clin Exp Ophthalmol* 241:518-521
- Walter P, Widder R, Lueke C, Koenigsfeld P, Brunner R (1999) Electrophysiological abnormalities in age-related macular degeneration. *Graefe's Arch Clin Exp Ophthalmol* 237:962-968
- Wang F, Jakobsen K, Smith W, Mitchell P (2004) Refractive status and the 5-year incidence of age-related maculopathy: the Blue Mountains Eye Study. *Jakobsen KB, Smith W, Mitchell P* 32:255-258

- Wang J, Foran S, Smith W, Mitchell P (2003a) Risk of age-related macular degeneration in eyes with macular drusen or hyperpigmentation. The Blue Mountains Eye Study Cohort. *Arch Ophthalmol* 121:658-663
- Wang J, Klein R, Smith W, Klein B, Tomany S, Mitchell P (2003b) Cataract surgery and the 5-year incidence of late-stage age-related maculopathy. *Ophthalmology* 110:1960-1967
- Wässle H, Boycott B (1991) Functional architecture of the mammalian retina. *Physiol Rev* 71:447-472
- Wässle H, Grünert U, Martin P, Boycott B (1994) Immunocytochemical characterization and spatial distribution of midget bipolar cells in the macaque monkey retina. *Vision Res* 34:561-579
- Watson T, Haegerstrom-Portnoy G (2004) Can the multifocal electroretinogram predict progression of age related maculopathy? 2004 Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO):Abstract 3086
- Webster A, Heon E, Lotery A, Vandenburg K, Casavant T, Oh K, Beck G, Fishman G, Lam B, Levin A, Heckenlively J, SG. J, Weleber R, Sheffield V, Stone E (2001) An analysis of allelic variation in the ABCR4 gene. *Invest Ophthalmol Vis Sci* 42:1179-1189
- Weeks D, Conley Y, Tsai H, Mah T, Schmidt S, Postel E, Agarwal A, Haines J, Pericak-Vance M, Rosenfeld P, Paul T, Eller A, Morse L, Dailey J, Ferrell R, Gorin M (2004) Age-related maculopathy: a genomewide scan with continued evidence of susceptibility loci within the 1q31, 10q26, and 17q25 regions. *Am J Hum Genet* 75:147-189
- Weih L, VanNewkirk M, McCarty C, Taylor H (2000) Age-specific causes of bilateral visual impairment. *Arch Ophthalmol* 118:264-269
- Weiter J, Delori F, Wing G, Fitch K (1985) Relationship of senile macular degeneration to ocular pigmentation. *Am J Ophthalmol* 99:185-187
- Weiter J, Delori F, Wing G, Fitch K (1986) Retinal pigment epithelial lipofuscin and melanin and choroidal melanin in human eyes. *Invest Ophthalmol Vis Sci* 27:145-152
- Wetzig P (1988) Treatment of drusen-related aging macular degeneration by photocoagulation. *Trans Am Ophth Soc* 86:276-290

- Wilder R (1995) Neuroendocrine-immune system interactions and autoimmunity. *Annu Rev Immunol* 13:307-338
- Wilson H, Schwartz D, Bhatt H, McCulloch C, Duncan J (2004) Statin and aspirin therapy are associated with decreased rates of choroidal neovascularisation among patients with age-related macular degeneration. *Am J Ophthalmol* 137:615-624
- Wing G, Blanchard G, Weiter J (1978) The topography and age relationship of lipofuscin concentration in the retinal pigment epithelium. *Invest Ophthalmol Vis Sci* 17:601-607
- Winkler B (1981) Glycolytic and oxidative metabolism in relation to retinal function. *J Gen Physiol* 77:667-692
- Winkler B (1983) Relative inhibitory effects of ATP depletion, ouabain and calcium on retinal photoreceptors. *Exp Eye Res* 36:581-594
- Witmer A, Vrensen G, Van Noorden C, Schlingemann R (2003) Vascular endothelial growth factors and angiogenesis in eye disease. *Prog Ret Eye Res* 22:1-29
- Wordehoff U, Palmowski A, Heinemann-Vernaleken B, Allgayer R, Ruprecht K (2004) Influence of cataract on the multifocal ERG recording-a pre- and postoperative comparison. *Doc Ophthalmol* 108:67-75
- Wu L (1991) The characteristic electroretinogram anomalies in age-related macular degeneration [Article in Chinese]. *Zhonghua Yan Ke Za Zhi* 27:197-199
- Wu S, Gao F, Maple B (2000) Functional architecture of synapses in the inner retina: segregation of visual signals by stratification of bipolar cell axon terminals. *J Neurosci* 20:4462-4470
- Wu S, Sutter E (1995) A topographic study of oscillatory potentials in man. *Vis Neurosci* 12:1013-1025
- Yamamoto S, Gouras P, Lopez R (1995) The focal cone electroretinogram. *Vis Res* 35:1641-1649
- Yamamoto S, Kamiyama M, Nitta K, Yamada T, Hayasaka S (1996) Selective reduction of the S cone electroretinogram in diabetes. *Br J Ophthalmol* 80:973-975
- Yannuzzi L, Ober M, Slakter J, Spaide R, Fisher Y, Flower R, Rosen R (2004) Ophthalmic fundus imaging: today and beyond. *Am J Ophthalmol* 137:511-524

- Yannuzzi L, Slakter J, Sorenson J, Guyer D, Orlock D (1992) Digital indocyanine green videoangiography and choroidal neovascularization. *Retina* 12:191-223
- Yanoff M, Fine B (1975) *Ocular Pathology. A Text and Atlas. Ocular Pathology. A Text and Atlas.* New York: Harper & Row:638
- Yeh T, Smith V, Pokorny J (1989) The effect of background luminance on cone sensitivity functions. *Invest Ophthalmol Vis Sci* 30:2077-2086
- Yoshii M, Yanashima K, Matsuno K, Wakaguri T, Kikuchi Y, Okisaka S (1998) Relationship between visual field defect and multifocal electroretinogram. *Jpn J Ophthalmol* 42:136-141
- Yoshii M, Yanashima K, Wakaguri T, Sakemi F, Kikuchi Y, Suzuki S, Okisaka S (2000) A basic investigation of multifocal electroretinogram: reproducibility and effect of luminance. *Jpn J Ophthalmol* 44:122-127
- Young R (1982) Early-stage abnormalities of foveal pi mechanisms in patients with retinitis pigmentosa. *J Opt Soc Am* 72:1021-1025
- Young R (1987) Pathophysiology of age-related macular degeneration. *Surv Ophthalmol* 31:291-306
- Yu D-Y, Cringle S (2001) Oxygen distribution and consumption within the retina in vascularised and avascular retinas and in animal models of retinal disease. *Prog Ret Eye Res* 20:175-208
- Yuodelis C, Hendrickson A (1985) A qualitative and quantitative analysis of the human fovea during development. *Vision Res* 26:847-855
- Zack D, Dean M, Molday R, Nathans J, Redmond T, Stone E, Swaroop A, Valle D, Weber B (1999) What can we learn about AMD from other retinal diseases? *Mol Vis* 5:30. Review.
- Zacks D, Ezra E, Terada Y, Michaud N, Connolly E, Gragoudas E, Miller J (2002) Verteporfin photodynamic therapy in the rat model of choroidal neovascularization: angiographic and histologic characterization. *Invest Ophthalmol Vis Sci* 43:2384-2391
- Zarepari S, Reddick A, Branham K, Moore K, Jessup L, Thoms S, Smith-Wheelock M, Yashar B, Swaroop A (2004) Association of apolipoprotein E alleles with susceptibility to age-related macular degeneration in a large cohort from a single center. *Invest Ophthalmol Vis Sci* 45:1306-1310
- Zelev A, Vingrys A (2005) Cathode-ray-tube monitor artefacts in neurophysiology. *Journal of Neuroscience Methods* 141:1-7

- Zhang C, Baffi J, Cousin S, Csaky K (2003) Oxidant-induced cell death in retinal pigment epithelium cells mediated through the release of apoptosis-inducing factor. *J Cell Sci* 42:1051-1056
- Zhao J, Frambach D, Lee P, Lee M, Lopez P (1995) Delayed macular choriocapillary circulation in age-related macular degeneration. *Int Ophthalmol Clin* 19:1-12