ELECTRORETINOGRAPHY IN UVEITIS

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Electroretinografie bij uveitis

(met een samenvatting in het Nederlands)

PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de
Universiteit Utrecht
op gezag van de
rector magnificus, prof.dr. H.R.B.M. Kummeling,
ingevolge het besluit van het college voor promoties
in het openbaar te verdedigen op

dinsdag 10 november 2020 des middags te 12.45 uur

door

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geboren op 9 februari 1991
te Amsterdam

Voor
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CHAPTER 1

GENERAL INTRODUCTION, AIMS AND SCOPE OF THIS THESIS

INTRODUCTION

Both anatomy, which describes the structure of organisms and their parts, and histology, which describes the structure of tissues, have helped scientists and doctors tremendously in their understanding of diseases. This understanding has led to new treatments and better prognoses for many patients. However, anatomy and histology do not always correspond with function.

The development and improvement of imaging techniques such as X-rays, magnetic resonance imaging (MRI), and optical coherence tomography (OCT) have improved the diagnostic capacities of doctors even more, because they help to visualize structures in a non-invasive, and 3D manner.

But, even though structures can tell us a lot about a tissue, function is often far more important for patients in their daily functioning. In ophthalmology, patients can surprise their physicians with a great visual function, despite a very abnormal scan. Unfortunately, the opposite may also be true. This thesis focusses on one aspect of function: the electrophysiological function of the retina in a disease called uveitis.

UVEITIS

Anatomy and anatomical classification of uveitis

When physiologists started to examine the eye, the ocular vascular bed appeared to them to have a some-what grape-like shape, after the sclera had been peeled off. They called it uvea: the Latin word for grape. The anatomical structure that we call the uvea nowadays, comprises the choroid, the ciliary body, the pars plana, and the iris. An inflammation of the uvea is called a uveitis. Interestingly, when ophthalmologists use the term uveitis, not only an inflammation of the uvea is implied; the adjacent structures, such as the retina, vitreous and anterior chamber may also show signs of inflammation.[1]

When ophthalmologists describe and classify uveitis, they try to determine the predominant site of the inflammation. This may be localized in the front, the middle, or in the back of the eye. Based on this anatomical localization, uveitis is called an anterior, intermediate, or a posterior uveitis. Sometimes no predominant site of inflammation can be defined: the entire eye appears to be inflamed. In such cases ophthalmologists call it a panuveitis.[1] Figure 1 illustrates this anatomical classification of uveitis.
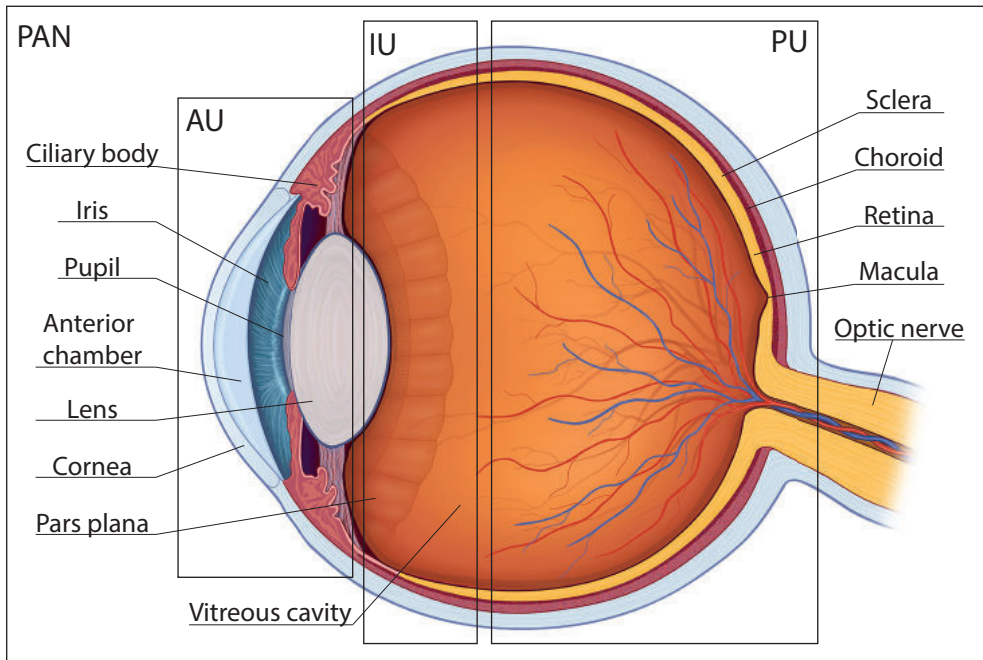
Figure 1: Illustration of the anatomy of the eye and it's relation to the different anatomical localizations of uveitis. Illustration recreated from: https://vsp.postclickmarketing.com/EyeAnatomyQuiz/eye-anatomy-review-dynamic-200WM-3388L1.html Abbreviations: AU = anterior uveitis, UI = intermediate uveitis, PU = posterior uveitis, PAN = panuveitis.

Causes of uveitis and associations with systemic diseases

The term 'uveitis' comprises about 30 disease entities. Each of these individual diagnoses have certain typical characteristics, but there are also overlapping aspects. This makes it sometimes quite difficult to distinguish the different uveitis diagnoses from one-another.

Uveitis may be caused by infectious agents, which may be identified by serology, or analyzing ocular fluids, or both.[2-4] However, many forms of uveitis are non-infectious. These non-infectious cases are often assumed to be auto-immune. However, in only about 5% of non-infectious uveitis cases, specific auto-antibodies can be found.[5] Many forms of uveitis have an association with systemic diseases including sarcoidosis, Bechterew, psoriasis, Behçet's disease, multiple sclerosis (MS) and juvenile idiopathic arthritis (JIA).[6-11] Sometimes the systemic disease is present prior to the uveitis, but in other cases the uveitis may be the first sign of the underlying systemic disease.

Uveitis also has several associations with certain human leukocyte antigen (HLA) types, including HLA-A29 (birdshot uveitis),[12,13] HLA-B27 (HLA-B27 associated

uveitis),[10,14] and HLA-B51 (Behçet's disease).[15] Lastly, there are several diseases which can mimic symptoms of uveitis, known as masquerade syndromes.[16–18]

One of the diseases that can be mistaken for uveitis are retinal dystrophies (RD), a group of genetic diseases that are characterized by progressive rod and cone dysfunction and degeneration. Unfortunately there is currently no treatment for RD. Therefore it is important to distinguish RD from uveitis, because uveitis can be be treated with immunomodulating medication to prevent retinal damage. This treatment is not useful in RD, so patients should not be exposed to the possible harmfull side-effects.[18]

Despite all the known causes and associations for uveitis, often no underlying cause for the inflammation can be found.[19,20] This thesis focusses on the electrophysiology of the retina in non-infectious uveitis, and includes all anatomical localizations of uveitis as well as all the underlying causes.

Epidemiology and treatment of uveitis

The prevalence of each of the different uveitis diagnoses is quite rare. However, as a group, uveitis is a much more common disease. The prevalence of uveitis varies around 85-204 cases per 100.000 cases, and the incidence is around 17-52 cases per 100.000 person-years.[21–23] The peak incidence of uveitis is around 25-44 years of age, but some forms of uveitis present typically in childhood, whereas others manifest later in life. Because uveitis affects many people in the working population, the visual impact is quite high: uveitis causes about 10-15% of the total blindness in the United States.[24–26]

Treatment of uveitis depends on the severity and underlying cause. In case of infectious uveitis, the underlying pathogen needs to be tackled. In case of non-infectious uveitis, the treatment is often focused on reducing the inflammation to prevent permanent damage to ocular tissue. Depending on the severity, localization and laterality of the inflammation, treatment can start with steroid eye droplets, streroid injections, or systemic medication.[20,27]

Systemic steroids are usually not administered for a long time, because the long-term treatment has negative side-effects. Therefore, if an ophthalmologist expects that long-term treatment is necessary, a disease-modifying antirheumatic drug (DMARD) (e.g. methotrexate, azathioprine, mycophenolate mofetil, mycophenolate sodium, cyclosporine, cyclophosphamide) or a biologic ((adalimumab or infliximab) is usually commenced as well.[20,28]

Complications of uveitis

Many complications can occur due to uveitis. Depending on the specific cause of uveitis, the incidence of these complications varies. Complications that can occur on the anterior segment of the eye include: cataracts, band keratopathy, corneal decompensation, ciliary body dysfunction, posterior synechiae and a pupillary occlusion.[29,30]

Complications to the posterior segment include cystoid macula edema (CME), glaucoma, vasculitis, retinal detachments, subretinal fibrosis, retinal neovascularization, retinal scarring, retinal atrophy and sometimes even phthisis bulbi.[29–32]

Several retinal changes can occur as well, which may result in retinal dystrophy like changes such as thinning of the retina, macula edema, and thinned vessels.[33–35] These changes can make it quite difficult to distinguish uveitis from retinal dystrophies.[18] But because uveitis can also lead to retinal atrophy, it would be useful to find early signs of retinal damage at a stage when it is still reversible.[32,36]

Monitoring uveitis activity and severity

Uveitis is monitored using multiple tests and examinations. What exact work-up is done, depends on the type and severity of uveitis. Generally it includes a best corrected visual acuity (BCVA), slit-lamp examination and funduscopy.[20]

The amount of inflammatory cells in both the anterior chamber and vitreous are graded according to the Standardisation of Uveitis Nomenclature (SUN) criteria. The amount of leakage of proteins due to the inflammation is graded in the anterior chamber as flare, and in the vitreous as vitreous haze.[1]

Ancillary tests that use imaging include optical coherence tomography (OCT), fluorescein angiography (FA) and indocyanine green angiography (ICG). The OCT is used to assess the structure of the retinal layers. It uses differences in interference between tissues to create images with a much higher resolution than ultrasound: OCT scans show the structure of the retinal layers in a manner which resembles histology. Figure 2 gives an overview of the different retinal layers which can be identified on OCT and their relation to histology.

Invasive imaging techniques that are frequently used include FA and ICG to identify vascular leakage or vascular occlusions. FA is used to evaluate the retinal vasculature, whereas ICG is used to visualize pathology in the choroid, the vascular layer beneath the retina.[37]

A frequently used physiological test is visual field analysis (VF). It is used to monitor and diagnose glaucoma, a frequent complication of uveitis, neuro-ophthalmologic disorders, but it can also be used to monitor progressive visual field loss in birdshot uveitis. Lastly, functional tests that are sometimes used are the electroretinogram (ERG) and visually evoked potentials (VEP).

ELECTRORETINOGRAM

The electroretinogram (ERG) is an objective test which assess the function of the retina. It measures the electrical signal of the cells of the retina in a response to light. In ophthalmology the ERG was originally used to help diagnose rare retinal disorders that affect retinal function. However, it is also sometimes used to monitor the progression of disease and to assess the effect of treatment.

Types of electroretinograms

Over the years several types of ERGs have been developed: the full-field ERG (ffERG) also known as Ganzfield ERG, the pattern ERG (PERG), and the multifocal ERG (mfERG).[38–40] The full-field ERG measures the response of nearly the entire retina in reaction to a flash of light. This flash of light is emitted from a so-called Ganzfield dome, which ensures an even illumination of the retina.

Another type of ERG, the PERG, is said to reflect the macular and ganglion cell function.[40] The mfERG is used to measure the function of different regions of the
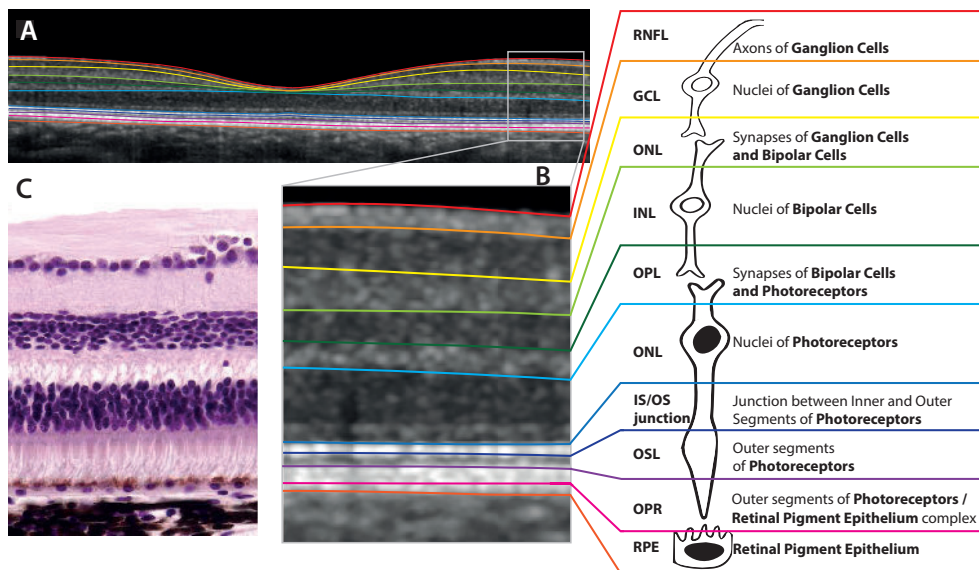
Figure 2: Optical coherence tomography scan of the retina in relation to retinal anatomy. A) OCT scan of the macula with segmentation lines indicating the boarders of several retinal layers. B) An enhancement of a part of OCT scan A. On the right of this enhancement is a scematic explanation of the different retinal cells of the segmented retinal layers. C) Histology biopt of a (different!) retina. Abbreviations: RNFL = Retinal Nerve Fibre Layer, GCL = ganglion cell layer, IPL = inner plexiform layer, INL = inner nuclear layer, OPL = outer plexiform layer, ONL = outer plexiform layer, IS/OS junction = inner segment outer segment junction, OLS = outer segment layer, OPR = outer segment photoreceptors/ retinal pigment epithelium complex, RPE = retinal pigment epithelium.

central retina, including the macula.[39] This thesis focusses on the full-field ERG, which is the most commonly used technique. For the rest of this thesis, when ERG is written, the full-field ERG is implied.

ERG waves and types of adaptations

There are several waves on an ERG curve that can be identified (Figure 2). The first is the negative a-wave. The a-wave represents the depolarization of the photoreceptors after a flash of light has been emitted.

The second wave is the (positive) b-wave. This wave is generated by the bipolar and Müller cells. On the rim of the ascending limb of the b-wave a 'saw-tooth' pattern can be observed. These are known as the oscillary potentials (OPs). The origin of these OPs remains unclear, some reports state that they reflect the amacrine cells, whereas others state that they are the result of bipolar cell activity.[41]

Besides the a-wave and b-wave, the c-wave and d-wave can be recorded as well. These represent the retinal pigment, and the OFF-center bipolar cells respectively. However, to record these waves, a much longer recording time is required.[42,43] Therefore, they are not routinely recorded and are not a subject of this thesis.

Lastly, a more recent aspect of the ERG was identified: the photonegative response (PhnR).[44] The PhnR can be observed after the b-wave. It is the negative reflection of the ERG curve which occurs after the positive b-wave. The PhnR reflects ganglion cell function and may be useful for detecting progression of glaucoma.[45] The PhnR is not a subject of this thesis, because its identification is not a part of our local ERG protocol.

When the eyes are adapted to light, the ERG response is primariliy cone driven (photopic response). When the eyes are adapted to the dark, the response to dim (weak) flashes is rod driven (scotopic response). As the flashs strengths become stronger (i.e the light flashes become brighter), a combined rod/cone response is recorded (mesopic response).

ISCEV standard and extended ISCEV protocols

To ensure that the results of electrophysiological research of vision would be better comparable, the International Society for Clinical Electrophysiology of Vision (ISCEV), has created multiple guidelines to record ERGs, as well as other electrophysiological tests.[38–40,46–48] Nowadays, virtually all scientific papers that report ERGs adhere to these standards.

Regarding the ERG, the ISCEV standard states the requirements for the equipment, flash strengths, background illumination, and the adaptation times for both
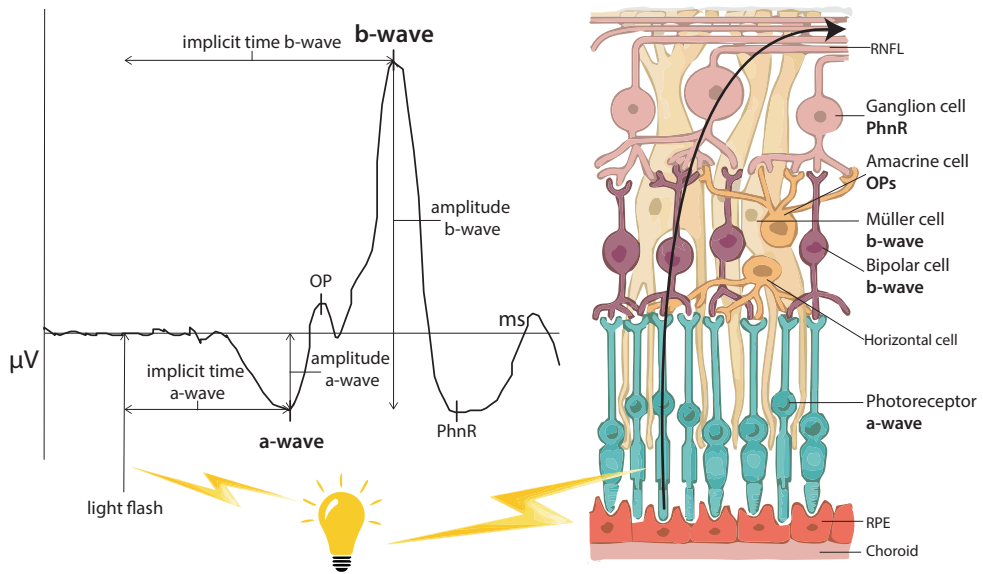
Figure 3: Explanation of ERG curves in relation to retinal cells. Abbreviations: ERG = electroretinogram, ms = miliseconds, µV = microvolt, OP = ocillary potential, PhnR = photonegative response, RNFL = retinal nerve fibre layer, RPE = retinal pigment epithelium.

dark adaptation (DA) and light adaptation (LA). The guidelines are updated regularly and with each update the writers try to ensure that this is done in such a manner that research can still be compared, even when a previous ISCEV version was adhered to. This implies that flash strengths are added, but not removed.[49]

Besides the 'basic' ISCEV standards, which state the minimum requirements for an ERG (DA 0.01 (rod response), DA 3.0 and 10.0 cds/m^2 (combined rod/cone response), LA 3.0 cds/m^2 (cone response) and 30 Hz (cone response)), ISCEV has also created multiple extended protocols. These extended protocols are mostly used in a scientific setting. However, in several Dutch hospitals, including the University Medical Center Utrecht (UMCU) and Bartiméus diagnostic Centre for complex visual disorders in Zeist, an extended protocol is recorded as standard practice. Recently new extended protocols were published. Many of the flash strengths of the extended protocol that was used in this thesis overlap with these new extended protocols, but not all.[50,51] Figure 4 shows an example of ERG curves of a healthy control of the extended protocol that was used in this thesis.

The ISCEV standards also describe how reference values should be obtained. It is important to have local reference values, because the type of equipment, type of electrodes, and local protocols can all influence the recorded ERG responses. Regarding amplitudes, the ISCEV standard states that amplitudes below the 5th percentile should be considered abnormal. Implicit times are considered abnormal

if they are above the 95th percentile. These one-sides values are probably chosen because most diseases that affect the ERG result in either reduced amplitudes or increased implicit times, or both. Increased amplitudes or reduced implicit times are rarely seen in case of pathology. Interestingly, the ISCEV standard does not describe how much ERG parameters need to be changed to be defined as significantly changed, which is necessary for monitoring purposes.[38]

Different types of electrodes

To record an ERG several electrodes are necessary: a common, or ground electrode, a reference electrode, and an active electrode which may contact the cornea, bulbar
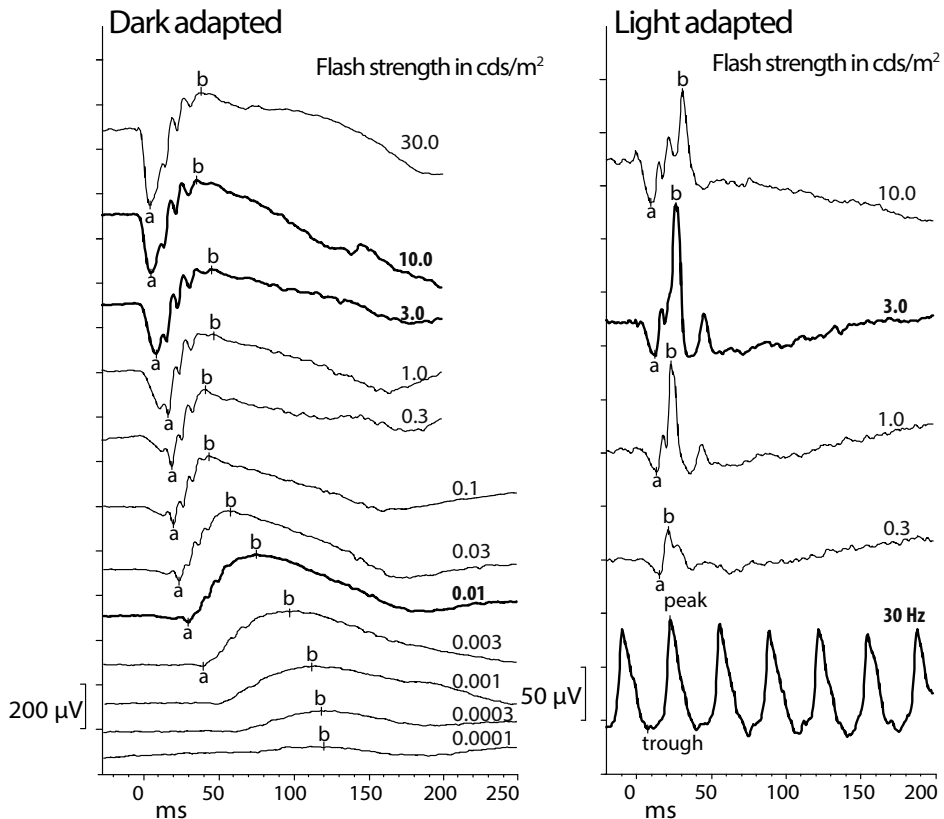
Figure 4: Example of ERG curves of a healthy control of the dark adapted and light adapted ERG of the different flash strengths. Recording time of the first seven flash strengths of the dark adapted ERG are 250ms. Those of the other flash strengths are 200ms. The standard ISCEV flash strengths (Dark adaptation: 0.01 (rod response), 3.0 and 10.0 cds/m^2 (combined rod/cone response) Light adaptation: 3.0 cds/m^2 (cone response) and 30 Hz (cone response)) are indicated in bold. Abbreviations: ERG = electroretinogram, cds/m^2 = candela · seconds/squared meters, ms = milliseconds, µV = microvolts, ISCEV = International Society for Clinical Electrophysiology of Vision.

conjunctiva, or skin of the lower eyelid. Over the years multiple types of active electrodes have been developed. Each has their own advantages and disadvantages and none is determined as the gold standard by ISCEV.

Initially, the main goal of electro-physicists was to record as high amplitudes as possible. These are best recorded at the top of cornea.[52] Therefore, for a long time Burrey-Allen contactlens electrodes were the most often used electrodes. While they yield high amplitudes, the disadvantage of these electrodes is that they are quite uncomfortable for patients to wear. Also, there is a chance of damage to the cornea due to scratching.

To increase patient comfort, several other electrodes were developed. One of these is the Dawson-Trick-Litzkov (DTL) electrode. In this thesis all ERG recordings were performed with DTL electrodes. When the DTL was first introduced, its developers stated that it was possible to use it without a local anesthetic.[53] However, it is our experience that it is advisable to use some local anesthetics to ensure a better patient comfort during the ERG recording. Besides a better patient comfort, the DTL electrode is usually disposable, which decreases the possibility of an infection. Lastly, with DTL the chance of significant damage to the cornea is small.

A disadvantage of the DTL electrode is that it can shift its position during an ERG recording. The position of the DTL can affect the recorded ERG response. In **chapter 6** we will look further into the effects of DTL electrode position and the ERG.

Factors other than disease that can influence the ERG

Besides retinal diseases, many other factors can influence the recorded ERG as well. Some of these factors can be (easily) addressed by adhering to the ISCEV standards. These include the duration of dark- and light adaptation, flash strength and pupil size.[38]

Light and dark adaptation appear to have an exponential effect on the ERG responses. In the first minutes most of the effect is reached, thereafter additional adaptation time yields only slightly larger responses.[54] The standard adaptation time for dark adaptation is 20 minutes, for light adaptation 10 minutes.

Several patient specific factors can also significantly affect the recorded ERG response, but these are less important when the ERG is used for monitoring purposes, because they are expected to remain stable over the sessions. Examples of these factors are axial length, ocular pigmentation and gender.[55–58]

Pupil size affects how much light can reach the retina. If the amount of light that can reach the retina is reduced, this should be taken into account when an ERG is reviewed. Therefore, a recorded ERG with a small pupil size may correspond to a weaker flash strength than the one which was used. Pupil sizes were therefore recorded in the prospective studies of this thesis.

Other factors can influence the recorded response, but are more difficult to address, include: DTL position, media opacities, age and even the time of day.[59–64] Uveitis can cause several types of media opacities, including corneal keratopathy, cataracts, flare, cells in the anterior chamber, vitreous cells, and vitreous haze.[1,29] These can all reduce the amount of light which reaches the retina. Posterior synechea, which can prevent the pupil form reaching full mydriasis can have a similar effect.[43] However, these media opacities will often only slighty reduce the amount of light that reaches the retina, but they will all have a larger effect on light scattering. Therefore, they will have a much larger influence on the mfERG than the ffERG. However, they can all have an effect on the ERG. Therefore, media opacities are always taken into consideration in the different chapters of this thesis.

LITERATURE ABOUT ELECTRORETINOGRAPHY OF UVEITIS

The role of the ERG is unclear for most forms of uveitis but the ERG may be used for monitoring birdshot uveitis, because some studies indicate that the 30 Hz flicker response of the ERG is a very sensitive marker to determine if systemic medication may be tapered.[65,66] Also, there are some reports that state that the ERG is useful to determine the activity of the inflammation in birdshot.[66] The ERG abnormalities in birdshot uveitis may improve after the inflammation has become inactive, but there are also reports that the ERG changes may be permanent.[65,67–72] There are indications that the ERG is helpful in predicting visual outcome in birdshot, but not all studies found a significant association.[71,72]

However, all studies that investigate the ERG in uveitis were conducted with relative small sample sizes and often a comparison with other diagnostic tests was not made. This makes it difficult to assess the true value of the ERG in uveitis. Therefore, the ERG is not a part in the standard diagnostic work-up in the University Medical Center Utrecht. Also, the ERG is not always correlated to other tests that monitor disease activity, and its measurement takes up a long period of time. Lastly, the true value of the ERG for monitoring is not elucidated.[69,73]

However, ERG changes in uveitis are not restricted to birdshot uveitis. There are many reports of ERG changes in different forms of uveitis.[74] Table 1 gives an overview of the different types of ERG and mfERG abnormalities that are described in uveitis. Many of these studies are case series, or studies conducted with small sample sizes. Depite the many reports of ERG abnormalities in uveitis, and the few reports that the ERG may be usefull for monitoring or prognosis, to our knowledge the ERG is not routinely used to monitor these uveitis entities.

Table 1: overview of ERG abnormalities that are described in literature (continued on next page)

| | Electoretinogram | | | Can the ERG improve? | mfERG |
| | Type of ERG abnormalities | | | | |
	Amplitudes Reduced?	Implicit times Prolonged?	Other		
AMPPE	Yes: - Photopic a-wave and b-wave [77] - Scotopic b-wave [77]			No[77]	-
AZOOR	Yes: - 30Hz flicker[78] - Photopic[78,79,96–102] - Scotopic[78,79,96–102] - Mesopic[78] - Undetectable[79,98,101] - Electronegative[97,102]	Yes: - 30 Hz flicker [79,98,101,102] - Photopic[79] - Scotopic[79]	- Can correlate to VF loss[97] - OPs affected[102] - b/a wave ratio normal[79]	Yes [79,98]	- Abnormal[78] - Corresponds to VF defects[78,101]
Behcet	Yes: [76,103,104] - b-wave and scotopic first affected[103,104]	Yes:[76]	- 'ERG abnormal'[105,106] - Can predict postoperative BCVA after cataract surgery[76] - OPS abnormal[105,106]	- No[75]	- Abnormal; amp. more affected than IT - Can improve[107]
Birdshot	Yes: - 30 Hz flicker[65,69,71,72,108–112] - Photopic [65,68,69,71,108,111,113,114] - Scotopic[65,67–69,108,109,111,113–115] - Mescopic [65,67,69,70,108,109,111,115–117] - Electronegative[65,116] - Almost non-recordable [68,109,111]	Yes: - 30 Hz flicker [65,67,118,69–72,108,110–112] - Photopic[71,108,111,113,117] - Scotopic[111,113,114,117] - Mesopic[108,109,111]	- OPs abnormal[68,114,115] - 30 Hz useful for visual prognosis[72,108,111,118] - Can deteriorate [65,68–70,111,112,119] - Doesn't always improve after treatment[108,112,120]	- Yes [65,67–70,108,111,112]	- Abnormal[121] - Correlated to BCVA and color vision[122] - Can improve or deteriorate[121]
Fuchs	Yes: - 30 Hz flicker[95] - Scotopic b-wave and a-wave[95]	Yes: - 30 Hz flicker[95] - Scotopic b-wave and a-wave[95]	- OPs reduced[95] - Normal[123]	-	-
IU	Yes: - 30 Hz flicker response[89,124] - Photopic b-wave[124] - Scotopic b-wave[124] - Mesopic b-wave and a-wave[124] - Nonrecordable[124]	Yes: - 30 Hz flicker response[89,124] - Photopic b-wave[124] - Mesopic b-wave and a-wave[124]	- OPs reduced[89,124] - Amplitude 30 Hz flicker and DA b-wave supernormal[89,124]	-	-
MEWDS	Yes[80,98,125–128] -30 Hz flicker[83] - Photopic[81,83] - Scotopic[81,83] - Mesopic[81] - Almost nonrecordable[83]	Normal[125]	-'ERG abnormal' [82,128,129] - OPs reduced[130]	Yes[83]	- Abnormal - Corresponds to VF[80,127,131,132] - Can improve[127]

Table 1 (continued)

	Electroretinogram Type of ERG abnormalities			Can the ERG improve?	mfERG
	Amplitudes Reduced?	Implicit times Prolonged?	Other		
MS			MS without uveitis: IT prolonged and amp. reduced[133]	-	
MFC			- 'Abnormal ERG'[134]	-	- Amp. abnormal[127] - Can improve[127]
POHS	Yes: -Photopic[98] - Scotopic[98]	-			
Sarcoid	- Borderline scotopic b-wave[88]	Yes: - Photopic b-wave[88] - Scotopic b-wave[88]	- 'Abnormal ERG'[135]	-	Normal[136]
Syphilis	Yes[137]: - 30 Hz flicker[94,138] - Photopic unrecordable[138] - Scotopic[94,138]	Normal[138]		Yes[94]	-P1 amp. reduced[137] - IT prolonged.[93] - nonrecordable[94,137] - Can improve,[94] but recovery slow[93,137]
TB			- 'Abnormal ERG'[139]		
Toxoplasma	- Photopic and scotopic reduced but not outside ref values.[92]	Normal[92]			
VKH	Yes[85,143] - 'little ERG response'[140]	Yes[143]	- 'Abnormal ERG'[142] - OPs absent[141] - LA useful for predicting postoperative vision after cataract surgery[87]	Yes[86,87,140,141,143]	- Amp. and IT affected[84,144] - Can improve[144]

Overview of the different ERG and mfERG abnormalities in uveitis that are described in literature. If papers specify which amplitudes or implicit times are abnormal (a-wave, b-wave, DA, LA etc.), this is specified below 'yes'. If a paper specificly states that the implicit times were normal, this is noted as well, because often only amplitudes are mentioned. If papers state that the ERG was abnormal and do not specify which aspect of the ERG was abnormal (amplitudes, implicit times etc.) 'abnormal ERG' in noted in the column 'other'. Abbreviations: amp = amplitude, APMPPE = acute posterior multifocal placoid pigment epitheliopathy, AZOOR = acute zonal occult outer retinopathy, BCVA = best corrected visual acuity, DA = dark adaptation, ERG = electroretinogram, IT = implicit time, IU = intermediate uveitis, MS = multiple sclerosis, MFC = multifocal chorioiditis, mfERG = multifocal electroretinogram, MEWDS = multiple evanescent white dot syndrome, OPs = occilary potentials, POHS = presumed ocular histoplasmosis syndrome, TB = tuberculosis, VF= visual fields, VKH = Vogt-Koyanaga-Harada.

It remains unclear at what stage in the disease process ERG abnormalities develop and if they are reversible or permanent. In addition, the type of ERG abnormalities vary amongst the uveitis entities. Whether there is one type of ERG abnormality which frequently occurs in all uveitis entities, remains unclear.

In Behçet's disease, it is reported that the ERG does not improve, even when the inflammation becomes quiet.[75] But a low a-wave or b-wave amplitude may be useful to predict a poor outcome of cataract surgery.[76]

In other uveitis entities, such as in acute posterior multifocal placoid pigment epitheliopathy (APMPPE), multiple evanescent white dot syndrome (MEWDS) and acute zonal occult outer retinopathy (AZOOR), the ERG may either improve or deteriorate over time.[77–83] In Vogt Koyanagi Harada (VKH) syndrome the mfERG, especially the implicit time of the P1, may be useful to detect early retinal damage prior to peripapillary atrophy.[84] Interestingly, in late stage VKH the implicit times of the ERG appear to be preserved, whereas the amplitudes are decreased.[85] Amplitudes can improve after treatment, but unfortunately they often worsen after two years of onset.[86] Similar to Behçet's disease, the ERG in VKH can also be used to predict outcome after cataract surgery.[87]

Abnormal implicit times have also been described in sarcoidosis and intermediate uveitis.[88,89] In childhood uveitis, ERG abnormalities are described with both amplitudes and implicit times being abnormal, even in the case of anterior uveitis.[90] In older studies a 'supernormal ERG' has also been decribed; this usually indicates an abnormally large amplitude of the b-wave, but cut off points of this supernormal b-wave are not provided. In most articles the supernormal b-wave is seen in the first active stage of the disease, after which the amplitude decreases either to within reference values, or below normal.[90,91] Lastly, ERG abnormalities have been described in infectious uveitis including toxoplasma chorioretinitis, ocular syphilis, and Fuchs heterochromic uveitis.[92–95]

Because the ERG is much more affected in retinal dystrophies than in uveitis, it may be used to distinguish uveitis from a retinal dystrophy. Using the ERG, several cases of retinal dystrophies were identified who were initially misdiagnosed as having an intermediate uveitis.[18]

AIMS AND SCOPE OF THIS THESIS

The main objective of this thesis was to investigate which ERG abnormalities occur in non-infectious uveitis. Because retinal atrophy occurs usually only after a long period of inflammation, a main objective was to investigate a possible correlation between the duration of uveitis and ERG abnormalities.

In **Chapter 2** we investigate this possible correlation by measuring the ERG in two groups: uveitis patients with a disease duration of < 1 year and > 5 years. We also correlate clinical parameters to the ERG to investigate what factors may predict ERG abnormalities.

In **Chapter 3** we look at the possible reversibility of the ERG abnormalities: a follow-up ERG was measured after 1 year in the group of patients with a short disease duration (1 year). We also correlate changes in ERG outcome to clinical parameters.

In **Chapter 4** we investigated possible correlations between retinal structures and retinal function. Therefore, we segmented the OCT scans and analysed the thickness of ten retinal layers. Next, we investigated differences in retinal layer thickness based on ERG outcome.

In **Chapter 5** we look further into pediatric uveitis patients with an intermediate or panuveitis who had an ERG to exclude retinal dystrophy. We investigated to what extent the ERG may be affected in childhood uveitis and what clinical factors are associated with abnormalities.

To investigate what the impact of an anterior uveitis is on retinal function, we describe the ERG of patients with a typical form of anterior uveitis: JIA-associated uveitis (**Chapter 6**).

In **Chapter 7** we investigate the effects of DTL position on the ERG. Due to blinking the DTL may shift which may be important when patients are uncomfortable, for instance due to photophobia in uveitis patients. We repeated the LA ERG twice in our uveitis patients to see if, and to what extent amplitudes, or implicit times, or both, were affected by the DTL position. We also investigated if there is an optimal DTL location for monitoring purposes.

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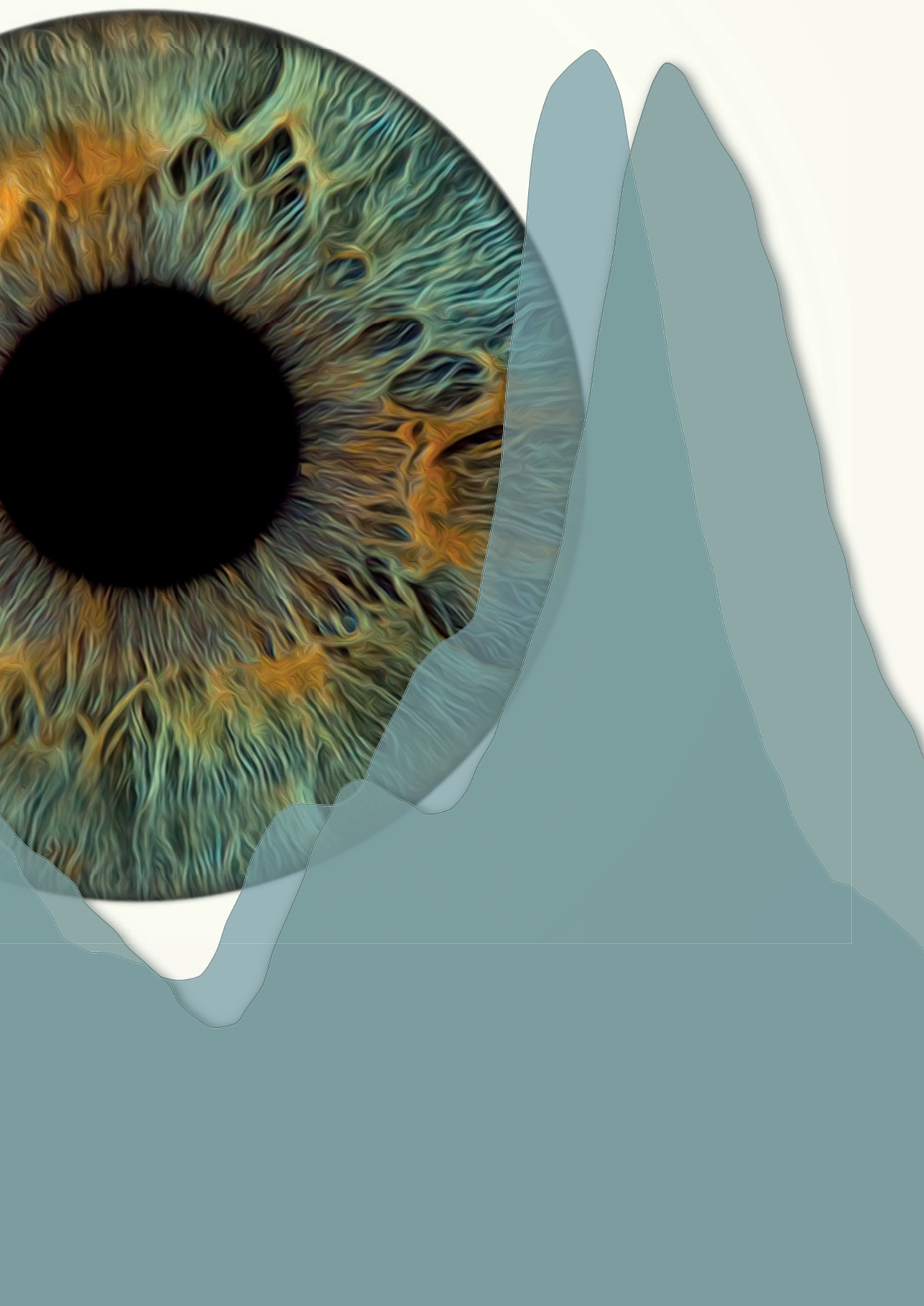
CHAPTER 2

PROLONGED CONE B-WAVE ON ELECTRORETINOGRAPHY IS ASSOCIATED WITH SEVERITY OF INFLAMMATION IN NONINFECTIOUS UVEITIS

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ABSTRACT

Purpose

This study sought to investigate retinal function in patients with noninfectious uveitis by using full-field electroretinography (ERG) and correlate the ERG to disease duration and severity of inflammation.

Design

Prospective cohort study.

Methods

Patients (n = 200) with noninfectious uveitis and a disease duration of < 1 year (group A, n = 80) and those with a disease duration of > 5 years (group B, n = 120) were included. ERGs of the total 355 uveitis eyes were measured according to an extended International Society for Clinical Electrophysiology of Vision protocol. ERG abnormalities were related to demographics and uveitis characteristics, including anatomical classification, duration of uveitis, severity of inflammation, best corrected visual acuity (BCVA), cystoid macular edema (CME), and the highest fluorescein angiography (FA) score during the course of the disease.

Results

ERGs showed abnormalities in 235 eyes (66.2%). The most frequent and pronounced ERG abnormality was a prolonged implicit time of the cone b-wave (151 eyes, 42.5%), which was associated with vitritis (p = .005), cells in the anterior chamber (p = .007), highest FA score (p = .011), age (p < .001), and pupil diameter (p < .001). BCVA was relatively good (0.05 LogMAR (interquartile range (IQR) 0.011 - 0.22)) and not associated with this ERG abnormality. There were no differences between the ERG abnormalities in group A and those in group B and no significant associations between ERG abnormalities and the anatomical classification or specific diagnoses.

Conclusions

The ERG results are frequently affected in cases of noninfectious uveitis of all anatomic subtypes, including anterior uveitis without apparent inflammation of the posterior segment. ERG abnormalities appear to be associated with the severity of inflammation from both the present and the past and therefore may be seen even when signs of retinal inflammation have disappeared.

INTRODUCTION

Noninfectious uveitis includes multiple diseases, some of which have an association with systemic diseases, whereas others are limited to the eye. Each of the individual diagnosis is relatively rare, and the prevalence of different subtypes varies around the world. In the United States the prevalence of uveitis is 121 cases per 100.000 adults.[1] Because uveitis frequently affects younger people, the social impact of the disease is high because it is responsible for approximately 10% of cases of blindness in the working population.[2]

Ocular inflammation may result in retinal changes, sometimes leading to retinal atrophy and functional loss.[3] Most of this retinal damage occurs after the inflammation has been present for several years. With new treatment options becoming available, ophthalmologists have more opportunities to treat this disease at an early stage before structural damage occurs to the ocular tissue.[4]

The electroretinogram (ERG) is an objective test which measures retinal function and which is mostly used to diagnose inherited or acquired retinal diseases. It may also be used for monitoring disease and to assess the extent of retinal damage, for instance, in ocular inflammation.[5] It is especially in early signs of retinal damage, prior to anatomical changes observed on optical coherence tomography (OCT), that the ERG would be of value in the care of patients.

In birdshot uveitis (BSCR) the ERG may be used for monitoring disease activity.[6–8] The 30 Hz flicker response appears to be the most sensitive parameter, but many other ERG abnormalities can occur as well.[7,8] Abnormal ERG test results have also been reported in other uveitis entities.[9] However, the value of measuring ERG in inflammatory eye disease has yet to be determined.

This prospective study was conducted to gain more insight into the effects of uveitis and disease duration on ERG results. Types of ERG abnormalities in uveitis and their relation to clinical parameters were investigated.

METHODS

Design and patient population

A prospective cohort study measuring a full-field ERG in 200 patients with a noninfectious uveitis was conducted. Patients with accompanying diabetic retinopathy, retinal dystrophy, family history of retinal dystrophy, myopic degeneration, or severe media opacities were excluded. All patients were treated for uveitis at the University Medical Center Utrecht (UMCU), the Netherlands, and were ≥ 18 years old. Patients

were enrolled between August 2016 and February 2019. The authors approached 457 patients, 189 declined, 62 met the exclusion criteria and 6 withdrew.

It was hypothesized that there would be an effect between short-term and long-term uveitis. To evaluate the possible effect of disease duration on the ERG, two groups were created. In group A (80 patients) an ERG was measured in the first year after disease onset, and in group B (120 patients) an ERG was measured after a disease duration of > 5 years. Disease onset was defined as the moment when an ophthalmologist first diagnosed uveitis. Patients with juvenile idiopathic arthritis (JIA) were excluded from both groups, because they were < 18 years of age at the start of disease and therefore could not be included in group A.

All patients were mentally competent and gave their consent to participate. This study was conducted in compliance with the ethical principles of the declaration of Helsinki. Ethical approval was requested and obtained from the Medical Ethical Research Committee of the UMCU prior to the start of the study.

ERG analysis

ERGs were performed on the same day subjects visited the outpatient clinic when best corrected visual acuity (BCVA) and uveitis activity also were recorded. The ERGs were measured according to the International Society for Clinical Electrophysiology of Vision (ISCEV) standards.[5] Dawson-Trick-Litzkow (DTL) electrodes were used as corneal electrodes and placed just below the cornea, following the lower eyelid. The use of DTLs is standard procedure in the Netherlands. Compared to contact lens electrodes, the DTL provides higher patient comfort and a lesser chance of infection, while still yielding reproducible responses.[10] An Espion E3 system with colordome stimulator (Diagnosys LLC, Cambridge, UK) was used for flash stimulation.

Measurements were made according to an extended ISCEV protocol, which is standard practice in the authors' hospital. An extended protocol is useful in monitoring disease, where subtle changes must be detected. This extended protocol consists of stimuli that increase with approximately 0.5 log units steps and range from 0.0001-30.0 cds/m^2 for dark-adapted (DA) ERGs and from 0.3-10.0 cds/m^2 for light-adapted (LA) ERGs. Also a standard a 30 Hz flicker response (LA) was recorded.

The ERGs of the patients were compared to those of healthy controls (n = 158; median age 47.7 years; interquartile range (IQR) 34.3 – 57.9). These reference ERGs were provided by the Rotterdam Eye Hospital and Bartiméus (Bartiméus Diagnostic Centre for complex visual disorders, Zeist) and measured with the same protocols on an Espion E2 system. Differences between the E2 and E3 system were within the measurement error. Also, the same equipment, protocols and standard operating

procedures were used and were supervised by the same physicist, to ensure that the reference data were representative. An ERG response was considered as abnormal if amplitudes were below the 5th percentile, or implicit times were above the 95th percentile, or both (see Supplemental table 6 for cut off values).

ERG response abnormalities were then categorized based on the measuring conditions (LA 0.3-10.0 cds/m^2 (single flash cone response), LA 3.0 cds/m^2 30 Hz flicker response (cone response), DA 0.01 cds/m^2 (single flash rod response), or DA 3.0-30.0 cds/m^2 (single flash combined rod/ cone response)) and which part of the response was abnormal (a-wave, b-wave, amplitudes and/or implicit times). In order for 1 of these categories to be defined as abnormal, the same type of ERG abnormality needed to be present in at least two consecutive flash strengths, except for the rod ERG, were the DA 0.01 cds/m^2 needed to be abnormal. So, if 2 ERG responses of consecutive flash strengths of the LA ERG showed a prolonged implicit time of the b-wave, the implicit time of the cone b-wave was defined as abnormal. B/a wave ratios were also evaluated.

Clinical parameters

Medical records were reviewed for age, sex, medical history, uveitis diagnosis, and anatomical localization of uveitis. The patient's age at diagnosis was recorded. Uveitis activity was graded according to the Standardization of Uveitis Nomenclature criteria: cell grade in the anterior chamber, cell grade in the vitreous, flare, and vitreous haze.[11] The presence of posterior synechiae, corneal clarity, lens clarity, macular lesions, snowballs, snow banking, vasculitis, glaucoma, and the appearance of the optic disc (hyperemia, edematous, pale) were also noted.

Additional information included whether patients were currently being treated with systemic steroids, disease-modifying antirheumatic drugs (DMARDS) (i.e. methotrexate (MTX), azathioprine, mycophenolate mofetil, mycophenolate sodium, cyclosporine, or cyclophosphamide), or biologicals (adalimumab or infliximab). Patients were also asked if they had been treated with a periocular/intraocular corticosteroid injection (betamethasone acetate 2.7 mg/bethamethasone, or sodium phosphate 3 mg periocular, up to 1 month prior to ERG. Triamcinolone 40 mg periocular, up to 3 months prior to ERG. Intravitreal dexamethasone 0.7 mg implant, up to 3 months prior to ERG).

All patients except 2 had undergone optical coherence tomography (OCT) (Cirrus HD OCT 5000, Zeiss Meditec, Dublin, California) examination on the same day as the ERG measurement, from which the presence of cysts (cystoid macular edema (CME), sub-retinal fluid, epiretinal membranes and central retinal thickness (CRT) were noted per eye.

If available, the mean deviation (MD) (n = 247 eyes) was recorded from the most recent visual field analysis (median 5 months, IQR 0 - 17 months) before the ERG measurement.

Fluorescein angiograms (FA) (n=333 eyes) were scored by an experienced ophthalmologist (JdB) who was blinded regarding ERG results, using the FA scoring system of the Angiography Scoring for Uveitis Working Group (ASUWOG)[12] as an indication of the severity of previous inflammation. FAs with the highest overall score until ERG measurement (median 10 months, IQR 3 - 64 months) were used for statistical analysis. When FAs were performed on the same day as the ERG (n = 9 patients), there was at least 1 hour between the FA and ERG.[13]

Statistical analysis

For statistical analysis SPSS version 25.0 (SPSS Inc., Chicago IL) was used. Only uveitis eyes were included. First, ERG results were compared to reference values, and differences between groups A and group B were investigated. A Mann-Whitney *U* test was used for continuous variables and the Pearson chi squared test or the likelihood ratio for categorical variables. Using Tukey's fences k = 3, possible outliers were identified.

Second, the most characteristic ERG abnormality result was selected and its relationship to clinical parameters was determined by using the Pearson chi-square test or the likelihood ratio for categorical variables, and a Student t-test or a Mann-Whitney *U* test for continuous variables, depending on the normality of data. Normality was tested using Kolmogorov-Smirnov normality test. A Spearman rank correlation coefficient was used to find possible correlations among variables.

Third, a multiple imputation was performed to address missing values. Herein all statistically significant variables of the univariate analysis were included, as well as several auxiliary variables and the outcome variable. The automated program of SPSS was used, but continuous variables were restricted to avoid unrealistic imputed variables. The number of imputations was equal to the maximum percentage of missing data plus 1.

Fourth, a generalized estimating equation (GEE) analysis was performed using the imputed data to correct for paired sampling of eyes and possible collinearity of parameters. Imputed data were used because a GEE analysis excludes eyes with one or more missing values, which would have led to biased results. Ordinal variables such as the amount of cells were transformed into categorical values (i.e. in the case of vitritis) to avoid complicating the model with multiple dummy variables. *P* values below 0.05 were considered statistically significant. All tests were 2-tailed.

RESULTS

Patient characteristics

Table 1 shows an overview of patient characteristics of both group A and B. The median age at ERG performance was, as expected, higher in group B compared to group A (54.9 years of age vs 48.4 years of age respectively, P = .007). In both groups there were more females than males. In group B, both eyes were more frequently affected than those in group A. However, analysis of only patients with a bilateral uveitis yielded no different results. Also, patients in group B were more frequently treated with disease-modifying antirheumatic drugs and had more frequently glaucoma. No differences were observed between groups based on diagnosis or anatomical localization of uveitis.

Table 1. Patient characteristics.

	Group A (n=80)	Group B (n=120)	P-value
Age,[a] median [IQR]	48.36 [31.41, 59.38]	54.91 [41.99, 66.62]	0.007
Male gender, N (%)	29 (36.2)	32 (26.7)	0.199
Bilateral uveitis,[a] N (%)	47 (58.8)	112 (93.3)	<0.001
Localization, N (%)			0.446
Anterior	5 (6.2)	15 (12.5)	
Intermediate	9 (11.2)	16 (13.3)	
Posterior	40 (50.0)	57 (47.5)	
Panuveitis	26 (32.5)	32 (26.7)	
Diagnosis, N (%)			0.207
Behçet	1 (1.2)	4 (3.3)	
Birdshot	8 (10.0)	19 (15.8)	
chorioretinitis[b]	16 (20.0)	18 (15.0)	
unknown	35 (43.8)	43 (35.8)	
HLA-B27	5 (6.2)	9 (7.5)	
MS	0 (0.0)	7 (5.8)	
Sarcoidosis	14 (17.5)	16 (13.3)	
VKH	1 (1.2)	4 (3.3)	
Oral prednisone,[c] N (%)	34 (42.5)	41 (34.2)	0.297
DMARD,[c] N (%)	20 (25.0)	74 (61.7)	<0.001
Biologics,[c,d] N (%)	1 (1.2)	8 (6.7)	0.144

a) At day of electroretinogram measurement. b) Includes patients with chorioiditis/chorioretinits of unknown cause, multiple evanescent white dot syndrome (MEWDS), acute multifocal placoid pigment epitheliopathy (AMPPE) and presumed ocular histoplasmosis syndrome (POHS). c) Treatment at time of ERG. d) adalimumab or infliximab. Abbreviations: IQR = interquartile range, HLA = human leukocyte antigen, MS = multiple sclerosis, VKH = Vogt–Koyanagi–Harada disease, DMARD = Disease-modifying anti-rheumatic drugs (i.e. methotrexate, azathioprine, mycophenolate mofetil, mycophenolate sodium, cyclosporine, cyclophosphamide).

ERG abnormalities

ERG abnormalities occurred in 235 of 355 uveitis eyes (66.2%) (Table 2). The most frequent and characteristic ERG abnormality was a prolonged implicit time of the (single- flash) cone b-wave (151 of 355 eyes (42.5%)). An example of the cone ERG of a uveitis patient is shown in Figure 1 (A). On the right (B) an overview of the implicit time of the cone b-wave of all uveitis patients compared to healthy controls can be seen. Four eyes were identified as possible outliers for a prolonged cone b-wave. These were not excluded since they met the outlier criteria in only 1 of the 4 flash strengths, whereas the other 3 flash strengths were prolonged, but not to an outlier extent.

On a group level, the ERGs results from uveitis patients were significantly worse compared to healthy controls at almost all flash strengths. Amplitudes were lower and implicit times longer in uveitis patients compared to healthy controls (Supplemental figures 1-3 and Supplemental table 6). It was only in the DA ERG amplitudes of the lowest stimuli (DA 0.0001-0.003 cds/m^2) that no statistically significant differences could be found between uveitis patients and healthy controls (Supplemental figures 1-3, and Supplemental table 6).
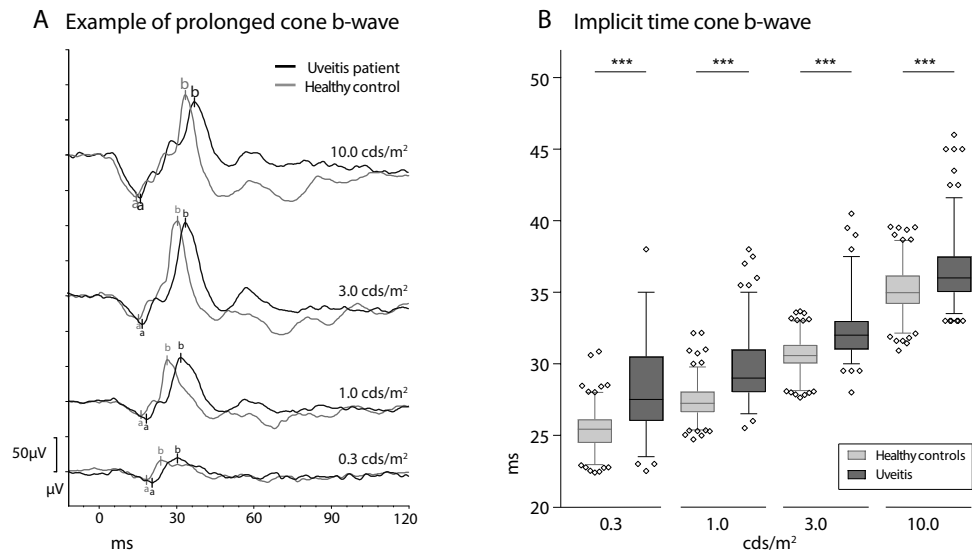
Figure 1: A) Example of a LA ERG of a patient with uveitis (black) and an ERG of an age matched control (gray). The single flash cone b-wave implicit times of the uveitis patient are prolonged; which is most pronounced at the lower stimulus strengths (LA 0.3 and 1.0 cds/m^2). B) Boxplots of the implicit time of the single flash cone b-wave of different flash strengths of uveitis patients compared to those of healthy controls. Statistical testing was performed using Mann-Whitney-U test. *** P-value of < 0.001. Abbreviations: LA = light-adapted, ERG = electroretinogram, cds/m^2 = candela · seconds/squared meters, ms = milliseconds, µV = microvolts.

Table 2. Type and amount of electroretinogram abnormalities in uveitis eyes

		All Eyes (N=355)	Group A (N=125)	Group B (N=230)	P-value
Overall		235 (66.2)	78 (62.4)	157 (68.3)	0.319
Single flash cone[a,b]		200 (56.3)	65 (52.0)	135 (58.7)	0.270
A-wave	Amplitude[b]	52 (14.6)	17 (13.6)	35 (15.2)	0.799
	Implicit time[b]	121 (34.1)	41 (32.8)	80 (34.8)	0.795
B-wave	Amplitude[b]	94 (26.5)	38 (30.4)	56 (24.3)	0.268
	Implicit time[b]	151 (42.5)	50 (40.0)	101 (43.9)	0.549
30 Hz flicker response[a,c]		167 (47.0)	54 (43.2)	113 (49.1)	0.338
	Amplitude[c]	121 (34.1)	36 (28.8)	85 (37.0)	0.152
	Implicit time[c]	123 (34.6)	39 (31.2)	84 (36.5)	0.374
Single flash rod[c]		123 (34.7)	41 (33.1)	82 (35.7)	0.711
B-wave	Amplitude[d]	92 (26.0)	30 (24.2)	62 (27.0)	0.661
	Implicit time[d]	55 (15.5)	17 (13.7)	38 (16.5)	0.587
Single flash rod cone[a,e]		154 (43.4)	52 (41.6)	102 (44.3)	0.699
A-wave	Amplitude[e]	71 (20.0)	25 (20.0)	46 (20.0)	<0.999
	Implicit time[e]	86 (24.2)	37 (29.6)	49 (21.3)	0.107
B-wave	Amplitude[e]	72 (20.3)	25 (20.0)	47 (20.4)	<0.999
	Implicit time[e]	54 (15.2)	20 (16.0)	34 (14.8)	0.880

Values are in N (%). a) Amount of eyes with one or more abnormalities (i.e. amplitude and/or implicit time of a-wave and/or b-wave). b) light-adapted 0.3-10.0 cds/m². c) light-adapted 3.0 cds/m². d) dark-adapted 0.01 cds/m². e) dark-adapted 3.0-30.0 cds/m². Abbreviations: cds/m² = candela · seconds/squared meters.

The implicit times of the (single flash) cone b-wave were significantly correlated to the peak implicit time of the 30 flicker response (Spearman's rho coefficient .620-.814, p-values < .001). We found no statistically significant differences in ERG abnormalities between group A and group B (Table 2).

Clinical characteristics

At time of ERG measurement, eyes in group A showed more frequently showed signs of an active inflammation than those in group B. The amount of cells in the vitreous was higher in group A than in group B (p < .001). These eyes also more frequently had CME on OCT (20.2% vs 8.8%,respectively; p = .004) as well as a hyperemic optic disc as observed with fundoscopy (23.6% vs 5.4%, respectively; p < .001) (Table 3).

Table 3. Inflammatory grade and other characteristics of group A and B at day of electroretinogram measurement

	Group A (n=125)	Group B (n=230)	P-value
Duration of uveitis in years, median [IQR]	0.59 [0.41, 0.74]	11.69 [8.04, 14.95]	<0.001
BCVA in LogMAR, median [IQR]	0.05 [-0.08, 0.15]	0.10 [0.00, 0.22]	0.010
Mean deviation VF,[a] median [IQR]	-1.88 [-3.69, -0.76]	-2.45 [-5.18, -0.58]	0.308
Glaucoma, N (%)	6 (4.8)	58 (25.2)	<0.001
CME on OCT, N (%)	25 (20.2)	20 (8.8)	0.004
Cells anterior chamber, N (%)			0.064
none	106 (86.2)	206 (92.0)	
trace	10 (8.1)	13 (5.8)	
1+	7 (5.7)	3 (1.3)	
2+	0 (0.0)	2 (0.9)	
Cells vitreous, N (%)			<0.001
none	57 (46.3)	147 (66.2)	
trace	26 (21.1)	51 (23.0)	
1+	26 (21.1)	19 (8.6)	
2+	8 (6.5)	5 (2.3)	
3	6 (4.9)	0 (0.0)	
Pale optic disc, N (%)	1 (0.8)	20 (9.0)	0.005
Hyperemic optic disc, N (%)	29 (23.6)	12 (5.4)	<0.001
Flare, N (%)			0.344
0	114 (92.7)	210 (93.8)	
1+	6 (4.9)	12 (5.4)	
2+	3 (2.4)	1 (0.4)	
3+	0 (0.0)	1 (0.4)	
Vitreous haze, N (%)			0.141
0	62 (50.4)	139 (62.3)	
.5+	42 (34.1)	60 (26.9)	
1+	17 (13.8)	23 (10.3)	
2+	2 (1.6)	1 (0.4)	
Lens/IOL opacities, N (%)	8 (6.5)	26 (11.7)	0.176
Steroid injection,[b] N (%)	7 (5.6)	7 (3.0)	0.370
Pupil diameter, median [IQR]	8.00 [7.00, 9.00]	8.00 [7.00, 9.00]	0.024

a) As measured on VF analysis (Humphrey) closest to ERG. b) Treated with periocular/intraocular corticosteroid injection (betamethasone acetate 2.7 mg/bethamethasonedisodiumphosphate 3 mg periocular; up to 1 month prior to ERG, triamcinolone 40 mg periocular; up to 3 months prior to ERG, intravitreal dexamethasone 0.7 mg implant; up to 3 months prior to ERG). Abbreviations: IQR = interquartile range, BCVA = best corrected visual acuity, MD = mean deviation, VF = visual field analysis, CME = cystoid macular edema, OCT = optical coherence tomography, CRT = central retinal thickness, IOL = intraocular lens.

Group B eyes had evidence of more severe activity in the past, compared to group A, based on FA scores previous to ERG measurement (Supplemental table 1). BCVA was worse in group B (median 0.10 LogMAR (IQR 0.00 - 0.22) vs median 0.05 LogMAR (IQR -0.08 - 0.15), p = .010) and the optic disc was more frequently pale compared to group A (9% vs 0.8%, respectively; p = .006). Although the total maximal FA score was not significantly higher in group B, the FA scores for CME (p < .001) and optic disc (p = .010) were higher in group B than group A (Supplemental table 1).

Interestingly, the prolonged cone b-wave was observed in uveitis at all anatomical locations (Supplemental table 2). Furthermore, the prolonged cone b-wave occurred in 38 eyes which were quiescent at time of ERG measurement.

Multiple imputation and GEE analysis

Because the prolonged implicit time of the cone b-wave is the most frequent and characteristic ERG abnormality, clinical parameters were studies as predictors for this abnormality.

Supplemental tables 2-4 show the results of a univariate analysis of possible predictors for a prolonged cone b-wave, based on patient characteristics and eye characteristics and FA scores. The following possible predictors were identified: age, BCVA, mean deviation on visual field analysis, pupil diameter, amount cells in the anterior chamber, amount of cells in the vitreous, vitreous haze, pale optic disc, CME on OCT, overall FA score and several subcategories of this FA score (optic disc, CME, vasculitis and capillary leakage). For further analyses, it was decided to use the overall FA score. Interestingly, an association was found with visual fields and a prolonged cone b-wave but not with glaucoma.

Most of these parameters were significantly correlated to each other (Supplemental table 5). However, Spearman's rho correlation coefficient was smaller than ±0.4 in all but one case. Only the correlation between BCVA and mean deviation on visual field analysis was higher than ±0.4 (Spearman's rho = -0.48, p < .001).

Next, a multiple imputation was performed using these possible predictors, the outcome variable (a prolonged cone b-wave) and possible auxiliary variables (sex, diagnosis and number of immunomodulating therapies). The percentage of missing values was < 5% for most variables. Except for VF, this was 30.4 %, 17.7 % for FA of the peripheral retina, and 5.4 % for FA of the posterior pole.

Table 4 shows the result of the GEE analysis. Because group A and B did not differ regarding ERG abnormalities, they were analyzed as one group. Several disease-specific factors were identified that were associated with a prolonged cone b-wave: vitritis, cells in the anterior chamber and FA score. However, age and pupil diameter were statistically significant as well.

Age was evaluated on a per-year basis, which implied that an odds ratio (OR) of 1.059 signifies an increase in OR for each year of age. This may appear small, but because the median age of our patient population was 53.4 years, it was relevant. This effect cannot be fully explained by a lower median age of our reference values (median 47.7 years) because when patients > 60 years of age were excluded in a separate analysis, age was still a significant predictor. To further investigate the effects of age, the mean implicit time of the cone b-wave was plotted against both uveitis eyes and reference values (Supplemental figure 4). The slope of the regression line of the implicit time versus age was much steeper in uveitis eyes at all flash strengths, which implies that there might be an additional effect of age in uveitis patients.

Because sex does not affect implicit times, a comparison was not made.[14] When effects of pupil size were analyzed, uveitis eyes with a small pupil size more frequently had prolonged implicit times of the cone b-wave than healthy controls (Supplemental figure 5).

Table 4: Clinical characteristics and their association with a prolonged cone b-wave

	OR	95% CI		P-value
Vitreous haze	0.757	0.232	2.477	0.646
Vitritis	3.031	1.410	6.516	0.005
Cells anterior chamber	4.060	1.457	11.310	0.007
FA score	1.088	1.020	1.161	0.011
Age	1.059	1.031	1.087	0.000
Duration of uveitis	1.007	0.967	1.048	0.733
Pupil diameter	0.619	0.478	0.801	0.000
Male gender	1.219	0.577	2.574	0.604
Pale optic disc	1.253	0.357	4.401	0.725
MD on VF	0.966	0.896	1.043	0.378
BCVA	1.278	0.486	3.362	0.619
CME	1.725	0.622	4.786	0.295

Results of a generalized estimating equation (GEE) analysis for possible predictors for a prolonged cone b-wave. Abbreviations: OR = odds ratio, CI = confidence interval, FA = fluorescein angiogram, MD = mean deviation, VF = visual field analysis, BCVA = best corrected visual acuity, CME = cystoid macula edema.

DISCUSSION

The results of this study showed that ERG abnormalities occur in two-thirds of eyes with uveitis. If the ERG result was abnormal, a prolonged cone b-wave was most common. In a multivariate model it was found that the severity of inflammation, as indicated by cells in the anterior chamber, vitritis, and the highest FA score during the disease course, were predictors for this cone b-wave prolongation. In a previous study of childhood uveitis, an association was also found between cone ERG abnormalities and vitritis and CME.[15] To the best of these authors' knowledge, there have been no other reports of the association between degree of inflammation and this specific cone ERG abnormality.

Generally, it is paramount to treat the inflammation instantly because a persistent inflammation has a worse visual outcome.[16,17] In the present study, quiescent uveitis eyes also had a prolonged cone b-wave, suggesting that not all ERG abnormalities are reversible and that previous inflammation can lead to permanent damage.

The significant association with the highest FA score during disease course but not disease duration also underlines the importance of quickly addressing and decreasing inflammation. Why treatment was not (inversely) associated with ERG abnormalities in the present study remains speculative. The influence of treatment on the ERG changes is the subject of a current longitudinal study. Interestingly, no association was found between ERG abnormalities and the duration of uveitis, which was hypothesized.

Also, no relationship was found between ERG abnormalities and the anatomical localization of uveitis or specific diagnosis. This means that not only intermediate, posterior uveitis or panuveitis but also anterior uveitis may lead to retinal involvement. Clinically, this finding is supported by the occurrence of CME and retinal changes in more severe cases of anterior uveitis. Even in Fuchs uveitis, in which CME is uncommon, ERG abnormalities have been reported.[18]

The present study included patients with presumed ocular histoplasmosis syndrome and multiple evanescent white dot syndrome. There is some debate whether or not these entities should be considered forms of uveitis. However, there are reports that they are auto-inflammatory conditions and similar to other chorioretinitis entities.[19,20] Furthermore, ERG abnormalities in these patients were similar to those observed in other uveitis patients, and exclusion of them did not yield different results (data not shown).

In addition to factors related to uveitis severity, age and pupil size were also significant predictors for a prolonged cone b-wave. Generally, implicit times increase with increasing age, so age was expected to be a predictor.[21,22] However, the slope of

implicit time versus age was much steeper in uveitis eyes than in reference values. This difference could not be explained by opacities of the lens or vitreous, indicating that there may be an additional uveitis effect on the ERG.

In healthy subjects, a smaller pupil size, which causes less light to fall on the retina, would result in a shorter implicit time of the cone b-wave. The strong association between smaller pupils and a more prolonged cone implicit time in the present cohort of uveitis patients therefore cannot be attributed to reduced retinal illumination but may be secondary to more active inflammation. If these eyes had reached full mydriasis, the implicit times would be even more prolonged.

The origin of the prolongation of the cone b-wave remains speculative. So far, no animal studies have investigated implicit times of the ERG in experimental autoimmune uveitis. Prolonged implicit times indicate impaired transmission of the electrical signals from the photoreceptors to the bipolar and Müller cells. One reason for impaired transmission might be damage to the synaptic structures. Evidence for synaptic damage was previously described in Behçet's disease, in which patients showed abnormalities of the outer plexiform layer on OCT.[23] However, in most eye diseases, a prolongation of the implicit time is usually combined with a reduced amplitude, whereas in uveitis, the implicit time alone is frequently affected.

Why the prolongation of the cone b-wave has not been more widely reported could be due to various reasons. First, cone implicit time abnormalities were most clearly seen at dimmer stimulus strengths, which are not measured with the standard ISCEV protocol and could therefore be missed. This may also explain why the 30 Hz flicker response was not always affected, since the flash strength corresponds with the brighter 3.0 cds/m^2.

Second, many reports of ERG abnormalities in uveitis focus on amplitudes. In line with previous reports, this study also found other numerous ERG abnormalities; on a group level, the ERGs of uveitis patients were worse than those of controls.

So far, it is unknown whether ERG parameters have prognostic value in uveitis. A relation was found between severity of inflammation and ERG changes, which has not been investigated in previous studies. Whether these ERG abnormalities are useful for monitoring therapy cannot be concluded from the present data, but they are subject of a current longitudinal study.

Additionally, a role for ERG in monitoring patients taking immunomodulating therapy has not been elucidated yet. Based on multiple reports with relative small sample sizes, it is said that, in birdshot uveitis, the implicit time of the 30 Hz flicker response is a sensitive marker for detecting early inflammation and to decide if immunotherapy is indicated or can be tapered.[6–8,24–26]

In Behçet's and Vogt Koyanagi Harada disease, the ERG results showed normalization after treatment and the LA ERG proved to be useful for predicting visual outcome after phacoemulsification.[27–29] Whether improvement in ERG results will occur in the group of patients with new-onset uveitis (group A), is subject to further study. Despite these reports, perhaps not all ERG parameters have been fully explored for their prognostic value. The present authors would recommend to using the extended ISCEV protocols in uveitis and to report all ERG parameters.

A limitation of this study is that the ERG was not always performed at an early stage of disease, before treatment was started. Because this study is not longitudinal, the true prognostic value of the ERG is unknown.

The possible effects of refractive error were not analyzed, because there were no refractive data of reference values. It was assumed that the refractive value of the uveitis cohort did not differ from that in the general population or the reference cohort, because (in contrast to retinal dystrophies) no correlation exists between high refractive error and having uveitis. Individuals with myopic degeneration were excluded from this study and from reference values. Another limitation is missing values, especially of visual field analysis, which was corrected by multiple imputation.

This study highlights the fact that the ERG is frequently affected in uveitis. The study found a characteristic prolongation of the cone b-wave that was associated with more severe inflammation in both the present and the past. Remarkably, even in eyes with anterior uveitis and in quiescent uveitis eyes, the ERG could be abnormal. Further research is needed to investigate to which extent ERG abnormalities are reversible and to explore the association between ERG abnormalities and visual prognosis.

ACKNOWLEDGEMENTS

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SUPPLEMENTAL MATERIAL

Supplemental table 1. Fluorescein angiography scores of uveitis eyes of group A and B (continued on next page)

	Group A (n=116)	Group B (n=200)	P-value
FA score, median [IQR]	4.00 [1.00, 8.00]	5.00 [1.00, 9.00]	0.373
Optic disc N (%)			0.006
0	40 (33.3)	73 (36.5)	
1	24 (20.0)	45 (22.5)	
2	20 (16.7)	53 (26.5)	
3	36 (30.0)	29 (14.5)	
Cystoid macula edema, N (%)			<0.001
0	68 (56.7)	81 (40.5)	
1	14 (11.7)	9 (4.5)	
2	7 (5.8)	20 (10.0)	
3	3 (2.5)	27 (13.5)	
4	28 (23.3)	63 (31.5)	
Vasculitis score, median [IQR]	1.00 [0.00, 3.00]	1.00 [0.00, 2.00]	0.635
Vasculitis posterior pole, N (%)			0.697
0	76 (63.3)	135 (67.5)	
1	12 (10.0)	23 (11.5)	
2	16 (13.3)	22 (11.0)	
3	16 (13.3)	20 (10.0)	
Vasculitis quadrants, N (%)			0.081
0	68 (65.4)	112 (59.6)	
1	8 (7.7)	26 (13.8)	
2	8 (7.7)	28 (14.9)	
3	5 (4.8)	4 (2.1)	
4	15 (14.4)	18 (9.6)	
Capp. leakage score, median [IQR]	0.00 [0.00, 1.00]	0.00 [0.00, 2.00]	0.139
Capp. Leakage posterior pole, N (%)			0.151
0	96 (80.0)	144 (72.0)	
1	11 (9.2)	18 (9.0)	
2	13 (10.8)	38 (19.0)	
Focal capp. leakage quadrants, N (%)			0.595
0	98 (94.2)	169 (89.9)	
1	3 (2.9)	9 (4.8)	
2	1 (1.0)	7 (3.7)	
3	1 (1.0)	2 (1.1)	
4	1 (1.0)	1 (0.5)	
Diffuse capp. leakage quadrants, N (%)			0.845
0	90 (86.5)	155 (82.4)	
2	3 (2.9)	7 (3.7)	
4	4 (3.8)	11 (5.9)	
6	0 (0.0)	1 (0.5)	
8	7 (6.7)	14 (7.4)	

Supplemental table 1. (continued)

	Group A (n=116)	Group B (n=200)	P-value
Non perfusion macula, N (%)	104 (100.0)	188 (100.0)	NA
Non perfusion posterior pole, N (%)	104 (100.0)	188 (100.0)	NA
Non perfusion quadrants, N (%)			0.162
0	102 (98.1)	188 (100.0)	
2	1 (1.0)	0 (0.0)	
3	1 (1.0)	0 (0.0)	
Neovascularization optic disc, N (%)	104 (100.0)	188 (100.0)	NA
Neovascularization elsewhere, N (%)			0.225
0	103 (99.0)	182 (96.8)	
1	0 (0.0)	5 (2.7)	
2	1 (1.0)	1 (0.5)	
Pinpoint leakage, N (%)			0.059
0	98 (94.2)	184 (97.9)	
1	3 (2.9)	0 (0.0)	
2	3 (2.9)	4 (2.1)	
Pooling, N (%)			0.059
0	100 (96.2)	185 (98.4)	
1	3 (2.9)	0 (0.0)	
4	1 (1.0)	3 (1.6)	

Fluorescein angiograms (FA) were scored using the Fluorescein angiographic scoring system of the Angiography Scoring for Uveitis Working Group (ASUWOG). FAs with the highest overall FA score until the ERG was recorded are shown. Abbreviations: FA = Fluorescein angiograms, N = number, IQR = interquartile range, capp. = capillary.

Supplemental table 2: Patient characteristics of uveitis eyes with a prolonged single flash cone b-wave

	Normal IT b-wave (n=112)	Prolonged IT b-wave (n=88)	P-value
Age,[a] median [IQR]	44.47 [32.77, 55.29]	60.93 [51.32, 70.13]	<0.001
Male gender, N (%)	36 (32.1)	25 (28.4)	0.678
Bilateral uveitis,[a] N (%)	84 (75.0)	75 (85.2)	0.109
Localization, N (%)			0.862
Anterior	13 (11.6)	7 (8.0)	
Intermediate	14 (12.5)	11 (12.5)	
Posterior	53 (47.3)	44 (50.0)	
Panuveitis	32 (28.6)	26 (29.5)	
Diagnosis, N (%)			0.138
Behçet	4 (3.6)	1 (1.1)	
Birdshot	14 (12.5)	13 (14.8)	
Chorioretinitis[b]	25 (22.3)	9 (10.2)	
unknown	42 (37.5)	36 (40.9)	
HLA-B27	8 (7.1)	6 (6.8)	
MS	5 (4.5)	2 (2.3)	
Sarcoidosis	13 (11.6)	17 (19.3)	
VKH	1 (0.9)	4 (4.5)	
Oral prednisone, N (%)	43 (38.4)	32 (36.4)	0.883
DMARD,[c] N (%)	48 (42.9)	46 (52.3)	0.237
Biologics,[c,d] N (%)	3 (2.7)	6 (6.8)	0.290

Patient characteristics with a prolonged single flash cone b-wave in one or both eyes. a) At time of ERG. b) Includes patients with chorioretinits of unknown cause, multiple evanescent white dot syndrome (MEWDS), acute multifocal placoid pigment epitheliopathy (AMPPE) and presumed ocular histoplasmosis syndrome (POHS). c) Treatment at time of ERG. d) adalimumab or infliximab. Abbreviations: IT = implicit time, ERG = electroretinography, IQR = interquartile range, HLA = human leukocyte antigen, MS = multiple sclerosis, VKH = Vogt–Koyanagi–Harada disease, IMT = immunomodulating therapy (i.e. systemic corticosteroids, methotrexate, azathioprine, mycophenolate mofetil, mycophenolate sodium, cyclosporine, cyclophosphamide, adalimumab and infliximab).

Supplemental table 3. Characteristics of eyes with a prolonged single flash cone b-wave

	Normal IT b-wave (n=200)	Prolonged IT b-wave (n=151)	P-value
Duration of uveitis, median [IQR]	7.56 [0.72, 12.37]	8.27 [0.73, 12.74]	0.221
BCVA, median [IQR]	0.00 [-0.08, 0.10]	0.15 [0.05, 0.30]	<0.001
Mean deviation VF,[a] median [IQR]	-1.66 [-3.17, -0.36]	-3.64 [-6.81, -1.61]	<0.001
Glaucoma, N (%)	32 (15.7)	32 (21.2)	0.232
Cystoid macula edema on OCT, N (%)	10 (5.0)	35 (23.3)	<0.001
Cells anterior chamber, N (%)			0.027
none	186 (93.0)	126 (85.7)	
trace	7 (3.5)	16 (10.9)	
1+	5 (2.5)	5 (3.4)	
2+	2 (1.0)	0 (0.0)	
Cells vitreous, N (%)			0.002
none	134 (67.0)	70 (48.3)	
trace	42 (21.0)	35 (24.1)	
1+	18 (9.0)	27 (18.6)	
2+	4 (2.0)	9 (6.2)	
3	2 (1.0)	4 (2.8)	
Pale optic disc, N (%)	6 (3.0)	15 (10.3)	0.010
Hyperemic optic disc, N (%)	23 (11.5)	18 (12.3)	0.946
Flair, N (%)			0.412
none	190 (95.0)	134 (91.2)	
faint	8 (4.0)	10 (6.8)	
moderate	2 (1.0)	2 (1.4)	
marked	0 (0.0)	1 (0.7)	
Vitreous haze, N (%)			0.007
none	131 (65.5)	70 (47.9)	
faint	46 (23.0)	56 (38.4)	
moderate	22 (11.0)	18 (12.3)	
marked	1 (0.5)	2 (1.4)	
Lens/IOL opacities, N (%)	15 (7.5)	19 (12.9)	0.138
Pupil diameter, median [IQR]	8.00 [8.00, 9.00]	7.00 [6.00, 8.00]	<0.001

a) Closest to ERG. Abbreviations: ERG = electroretinography, IQR = interquartile range, BCVA = best corrected visual acuity, IT = implicit time, MD = mean deviation, VF = visual field analysis, CME = cystoid macular edema, OCT = optical coherence tomography, CRT = central retinal thickness, IOL = intraocular lens

Supplemental table 4. FA scores of eyes with a prolonged single flash cone b-wave (continued on next page)

	Normal IT b-wave (n=204)	Prolonged IT b-wave (n=151)	P-value
FA score, median [IQR]	4.00 [0.00, 7.75]	7.00 [4.00, 11.00]	<0.001
Optic disc N (%)			0.008
0	76 (42.5)	37 (26.2)	
1	40 (22.3)	29 (20.6)	
2	33 (18.4)	40 (28.4)	
3	30 (16.8)	35 (24.8)	
Cystoid macula edema, N (%)			<0.001
0	111 (62.0)	38 (27.0)	
1	11 (6.1)	12 (8.5)	
2	13 (7.3)	14 (9.9)	
3	12 (6.7)	18 (12.8)	
4	32 (17.9)	59 (41.8)	
Vasculitis score, median [IQR]	0.00 [0.00, 2.00]	1.00 [0.00, 3.00]	0.002
Vasculitis posterior pole, N (%)			0.021
0	131 (73.2)	80 (56.7)	
1	15 (8.4)	20 (14.2)	
2	16 (8.9)	22 (15.6)	
3	17 (9.5)	19 (13.5)	
Vasculitis quadrants, N (%)			0.008
0	115 (67.6)	65 (53.3)	
1	13 (7.6)	21 (17.2)	
2	18 (10.6)	18 (14.8)	
3	8 (4.7)	1 (0.8)	
4	16 (9.4)	17 (13.9)	
Capp. leakage score, median [IQR]	0.00 [0.00, 0.75]	0.00 [0.00, 3.00]	<0.001
Capp. leakage posterior pole, N (%)			0.003
0	146 (81.6)	94 (66.7)	
1	15 (8.4)	14 (9.9)	
2	18 (10.1)	33 (23.4)	
Focal capp. leakage quadrants, N (%)			0.029
0	163 (95.9)	104 (85.2)	
1	3 (1.8)	9 (7.4)	
2	2 (1.2)	6 (4.9)	
3	1 (0.6)	2 (1.6)	
4	1 (0.6)	1 (0.8)	
Diffuse capp. leakage quadrants, N (%)			0.017
0	150 (88.2)	95 (77.9)	
2	6 (3.5)	4 (3.3)	
4	8 (4.7)	7 (5.7)	
6	1 (0.6)	0 (0.0)	
8	5 (2.9)	16 (13.1)	

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Supplemental table 4 (continued)

	normal IT b-wave (n=204)	prolonged IT b-wave (n=151)	P-value
Non perfusion macula, N (%)	170 (100.0)	122 (100.0)	NA
Non perfusion posterior pole, N (%)	170 (100.0)	122 (100.0)	NA
Non perfusion quadrants, N (%)			0.348
0	169 (99.4)	121 (99.2)	
2	0 (0.0)	1 (0.8)	
3	1 (0.6)	0 (0.0)	
Neovascularization optic disc, N (%)	170 (100.0)	122 (100.0)	NA
Neovascularization elsewhere, N (%)			0.152
0	166 (97.6)	119 (97.5)	
1	4 (2.4)	1 (0.8)	
2	0 (0.0)	2 (1.6)	
Pinpoint leakage, N (%)			0.474
0	166 (97.6)	116 (95.1)	
1	1 (0.6)	2 (1.6)	
2	3 (1.8)	4 (3.3)	
Pooling, N (%)			0.097
0	167 (98.2)	118 (96.7)	
1	0 (0.0)	3 (2.5)	
4	3 (1.8)	1 (0.8)	

Fluorescein angiograms (FA) were scored using the Fluorescein angiographic scoring system of the Angiography Scoring for Uveitis Working Group (ASUWOG). FAs with the highest overall FA score until the ERG was recorded are shown. Abbreviations: FA = Fluorescein angiogram, IT = implicit time, N = number, capp. = capillary.

Supplemental table 5: Spearman's rho correlation coefficient of possible predictors for a prolonged single flash cone b-wave

		Vitreous haze	Vitritis	Male gender	Pale optic disc	Cells ant. chamber	CME	FA score	Age	Duration of uveitis	Pupil diameter	MD on VF	BCVA
Vitreous haze	rho	1.00	0.23	0.08	0.01	0.05	0.12	0.08	0.08	-0.03	0.07	-0.07	0.04
	p-value		0.000	0.153	0.791	0.374	0.028	0.162	0.116	0.576	0.197	0.264	0.471
Vitritis	rho	0.23	1.00	0.02	0.00	0.23	0.17	0.16	0.03	-0.25	0.02	0.01	0.09
	p-value	0.000		0.714	0.952	0.000	0.001	0.008	0.538	0.000	0.652	0.873	0.079
Male gender	rho	0.08	0.02	1.00	0.06	-0.04	-0.01	0.04	0.06	-0.06	0.09	-0.02	0.02
	p-value	0.153	0.714		0.291	0.457	0.841	0.546	0.236	0.235	0.087	0.774	0.664
Pale optic disc	rho	0.01	0.00	0.06	1.00	0.04	-0.02	0.06	0.12	0.23	-0.14	-0.33	0.19
	p-value	0.791	0.952	0.291		0.515	0.645	0.314	0.032	0.000	0.008	0.000	0.000
Cells ant. chamber	rho	0.05	0.23	-0.04	0.04	1.00	0.04	0.00	-0.17	-0.04	0.00	-0.03	0.06
	p-value	0.374	0.000	0.457	0.515		0.417	0.979	0.002	0.426	0.960	0.618	0.281
CME	rho	0.12	0.17	-0.01	-0.02	0.04	1.00	0.31	0.22	-0.11	-0.19	-0.11	0.36
	p-value	0.028	0.001	0.841	0.645	0.417		0.000	0.000	0.042	0.000	0.084	0.000
FA score	rho	0.08	0.16	0.04	0.06	0.00	0.31	1.00	0.21	0.05	-0.16	-0.25	0.34
	p-value	0.162	0.008	0.546	0.314	0.979	0.000		0.000	0.390	0.008	0.000	0.000
Age	rho	0.08	0.03	0.06	0.12	-0.17	0.22	0.21	1.00	0.17	-0.33	-0.11	0.29
	p-value	0.116	0.538	0.236	0.032	0.002	0.000	0.000		0.001	0.000	0.075	0.000
Duration of uveitis	rho	-0.03	-0.25	-0.06	0.23	-0.04	-0.11	0.05	0.17	1.00	-0.15	-0.17	0.18
	p-value	0.576	0.000	0.235	0.000	0.426	0.042	0.390	0.001		0.005	0.006	0.001
Pupil diameter	rho	0.07	0.02	0.09	-0.14	0.00	-0.19	-0.16	-0.33	-0.15	1.00	0.17	-0.27
	p-value	0.197	0.652	0.087	0.008	0.960	0.000	0.008	0.000	0.005		0.007	0.000
MD on VF	rho	-0.07	0.01	-0.02	-0.33	-0.03	-0.11	-0.25	-0.11	-0.17	0.17	1.00	-0.48
	p-value	0.264	0.873	0.774	0.000	0.618	0.084	0.000	0.075	0.006	0.007		0.000
BCVA	rho	0.04	0.09	0.02	0.19	0.06	0.36	0.34	0.29	0.18	-0.27	-0.48	1.00
	p-value	0.471	0.079	0.664	0.000	0.281	0.000	0.000	0.000	0.001	0.000	0.000	

Abbreviations: ant. = anterior, CME = cystoid macular edema, FA = fluorescein angiography, MD = mean deviation, VF = visual field analysis, BCVA = best corrected visual acuity.

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Supplemental table 6: Cut off values and overview of reference values and uveitis patients (continued on next page)

		Cut off value	HC median [IQR]	Uveitis median [IQR]	P-value
Light adapted ERG	**A-wave amplitude**				
	0.3	-5.17	-12.35 [-17.01, -8.38]	-11.80 [-15.49, -8.13]	0.046
	1.0	-10.67	-21.28 [-26.93, -16.45]	-19.07 [-25.13, -13.80]	<0.001
	3.0	-21.31	-36.13 [-44.15, -29.10]	-29.59 [-37.73, -22.11]	<0.001
	10.0	-31.27	-52.58 [-63.13, -41.90]	-45.98 [-57.64, -35.24]	<0.001
	A-wave implicit time				
	0.3	19.65	17.64 [16.81, 18.50]	19.00 [18.50, 20.00]	<0.001
	1.0	17.55	15.87 [15.07, 16.50]	17.00 [16.50, 18.00]	<0.001
	3.0	16.16	14.98 [14.34, 15.44]	16.00 [15.00, 16.50]	<0.001
	10.0	15.12	13.91 [13.45, 14.45]	15.00 [14.50, 15.50]	<0.001
	B-wave amplitude				
	0.3	21.50	36.31 [28.86, 46.11]	32.19 [22.84, 40.91]	<0.001
	1.0	55.45	92.92 [76.76, 116.80]	74.12 [53.73, 96.21]	<0.001
	3.0	92.12	139.40 [116.28, 170.70]	116.60 [89.54, 151.05]	<0.001
	10.0	78.40	129.00 [105.60, 160.87]	117.20 [91.06, 144.20]	<0.001
	B-wave implicit time				
	0.3	27.62	25.43 [24.47, 26.10]	27.50 [26.00, 30.50]	<0.001
	1.0	29.32	27.24 [26.61, 28.05]	29.00 [28.00, 31.00]	<0.001
	3.0	32.57	30.56 [30.02, 31.33]	32.00 [31.00, 33.00]	<0.001
	10.0	38.16	34.96 [34.17, 36.17]	36.00 [35.00, 37.50]	<0.001
	30 Hz flicker response				
	Amplitude peak	52.37	88.35 [67.86, 110.53]	63.86 [46.11, 82.97]	<0.001
	Implicit Time Trough	23.16	20.34 [19.77, 21.60]	22.50 [21.00, 23.50]	<0.001
	Implicit Time Peak	30.38	27.31 [26.45, 28.31]	29.50 [28.00, 31.50]	<0.001
Dark adapted ERG	**A-wave amplitude**				
	0.0001		-5.36 [-10.00, -2.34]	0.00 [0.00, 0.00]	<0.001
	0.0003		-6.31 [-10.59, -2.60]	0.00 [-3.64, 3.37]	<0.001
	0.001		-7.72 [-13.32, -3.99]	-0.90 [-5.04, 3.27]	<0.001
	0.003		-10.82 [-18.36, -4.92]	-3.23 [-10.94, 0.71]	<0.001
	0.01		-19.76 [-29.95, -13.03]	-13.47 [-22.97, -3.99]	<0.001
	0.03		-38.58 [-52.41, -27.54]	-26.75 [-41.47, -15.70]	<0.001
	0.1		-63.94 [-84.69, -43.96]	-41.59 [-60.39, -27.88]	<0.001
	0.3		-115.90 [-148.40, -90.46]	-77.55 [-112.10, -52.44]	<0.001
	1.0		-182.85 [-228.60, -153.10]	-143.30 [-194.55, -107.45]	<0.001
	3.0	-131.15	-227.90 [-277.98, -188.20]	-178.60 [-227.45, -136.85]	<0.001
	10.0	-176.30	-266.80 [-328.55, -229.50]	-229.40 [-279.70, -178.30]	<0.001
	30.0	-177.93	-274.00 [-337.20, -234.05]	-240.20 [-290.45, -191.05]	<0.001

Cut off values were based on reference values. An amplitude was considered as abnormal if it was below the 5th percentile, for implicit times if it was above the 95th percentile. LA 0.3 - 10.0 cds/m² flash strengths were used for the single flash cone response. The LA 3.0 cds/m² for the 30 Hz flicker response (cone response). DA 0.01 cds/m² for the single flash rod response and DA 3.0 - 30.0 cds/m² for the single flash combined rod/cone response. In order to be defined abnormal, the same type of abnormality needed to be present in at least two consecutive flash intensities, except for the rod ERG, were the DA 0.01 cds/m² needed to be abnormal. Abbreviations: ERG = electroretinography, IQR = interquartile range, LA = light adapted, DA = dark adapted.

|	Cut off value	HC median [IQR]	Uveitis median [IQR]	P-value
A-wave implicit time				
0.0001		80.49 [76.15, 85.31]	80.00 [75.12, 82.38]	0.094
0.0003		74.19 [69.88, 76.96]	75.00 [71.00, 79.00]	0.015
0.001		61.21 [58.33, 64.40]	63.00 [60.00, 66.50]	<0.001
0.003		49.44 [46.76, 52.50]	52.00 [49.00, 55.00]	<0.001
0.01		39.17 [37.42, 40.67]	41.50 [39.50, 44.00]	<0.001
0.03		32.75 [31.35, 34.25]	34.50 [33.00, 37.00]	<0.001
0.1		28.35 [27.25, 29.25]	30.00 [28.50, 32.00]	<0.001
0.3		25.57 [24.80, 26.29]	27.00 [26.50, 29.00]	<0.001
1.0		23.21 [22.54, 23.88]	24.50 [24.00, 26.00]	<0.001
3.0	17.70	16.08 [15.44, 16.68]	17.50 [16.50, 18.00]	<0.001
10.0	15.53	13.21 [12.24, 14.07]	15.00 [14.00, 16.00]	<0.001
30.0	14.78	12.18 [11.01, 13.29]	13.00 [11.50, 14.00]	<0.001
B-wave amplitude				
0.0001		21.66 [15.12, 33.48]	16.32 [11.42, 24.06]	<0.001
0.0003		62.56 [42.33, 83.97]	32.88 [19.95, 52.29]	<0.001
0.001		131.65 [100.48, 170.25]	79.68 [51.60, 118.15]	<0.001
0.003		210.20 [170.83, 271.10]	147.90 [104.27, 203.80]	<0.001
0.01	169.14	282.30 [229.67, 349.78]	226.30 [164.90, 281.50]	<0.001
0.03		309.30 [252.05, 376.98]	265.95 [206.80, 322.88]	<0.001
0.1		309.10 [253.65, 382.10]	270.50 [210.85, 329.75]	<0.001
0.3		349.15 [284.58, 418.35]	299.30 [229.90, 359.00]	<0.001
1.0		384.50 [318.62, 475.15]	338.70 [267.75, 414.25]	<0.001
3.0	260.52	401.05 [333.13, 485.07]	350.00 [276.65, 413.45]	<0.001
10.0	286.91	414.50 [353.75, 507.90]	380.40 [299.20, 453.45]	<0.001
30.0	282.89	418.75 [344.52, 507.90]	379.20 [295.95, 448.70]	<0.001
B-wave implicit time				
0.0001		131.75 [124.99, 141.04]	125.50 [112.00, 134.00]	<0.001
0.0003		135.51 [130.78, 140.82]	134.00 [127.50, 139.50]	0.027
0.001		126.64 [120.23, 132.19]	127.00 [121.00, 132.50]	0.395
0.003		110.52 [105.14, 116.79]	112.00 [106.00, 119.00]	0.096
0.01	104.30	89.20 [84.38, 96.42]	93.00 [85.50, 101.00]	<0.001
0.03		75.88 [70.10, 82.97]	78.00 [73.00, 83.38]	<0.001
0.1		58.65 [53.65, 67.33]	63.00 [57.50, 71.50]	<0.001
0.3		49.56 [47.30, 52.83]	53.00 [50.50, 56.75]	<0.001
1.0		49.45 [47.11, 52.74]	50.50 [48.50, 54.50]	<0.001
3.0	58.09	51.77 [49.78, 54.25]	55.50 [52.50, 57.00]	<0.001
10.0	56.05	51.52 [49.22, 54.18]	55.00 [53.50, 56.00]	<0.001
30.0	55.14	49.32 [43.50, 52.08]	52.50 [51.00, 54.00]	<0.001
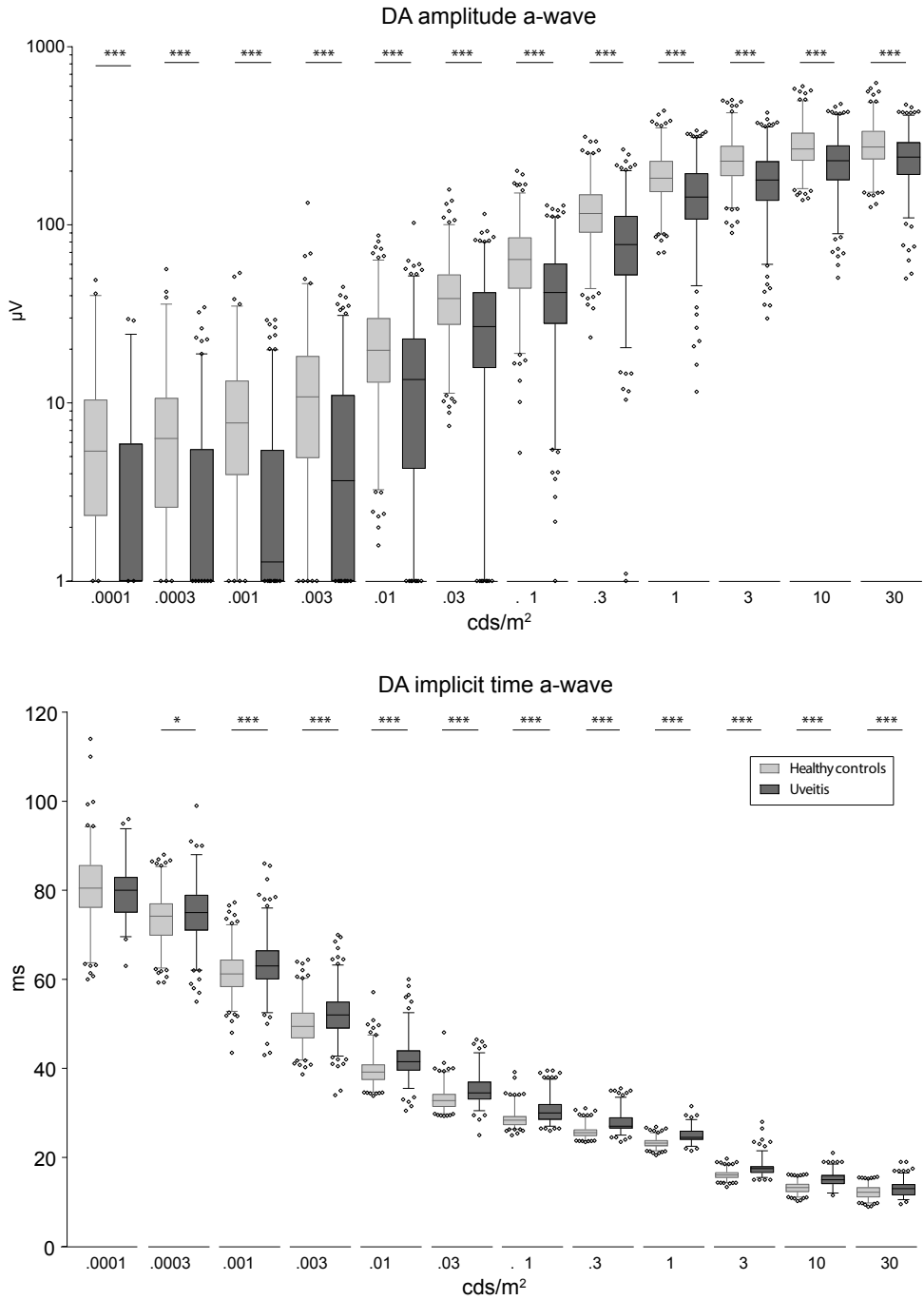
Dark adapted

Supplemental figure 1: Boxplots of the amplitudes and implicit times of different flash intensities of the (single flash) dark-adapted a-wave of uveitis patients compared to healthy controls. Statistical testing was performed using Mann-Whitney-U test. * P-value < 0.05. *** P-value of < 0.001. Abbreviations: cds/m² = candela · seconds/squared meters, DA = dark adapted, ms = milliseconds, µV = microvolts.

DA amplitude b-wave

DA implicit time b-wave
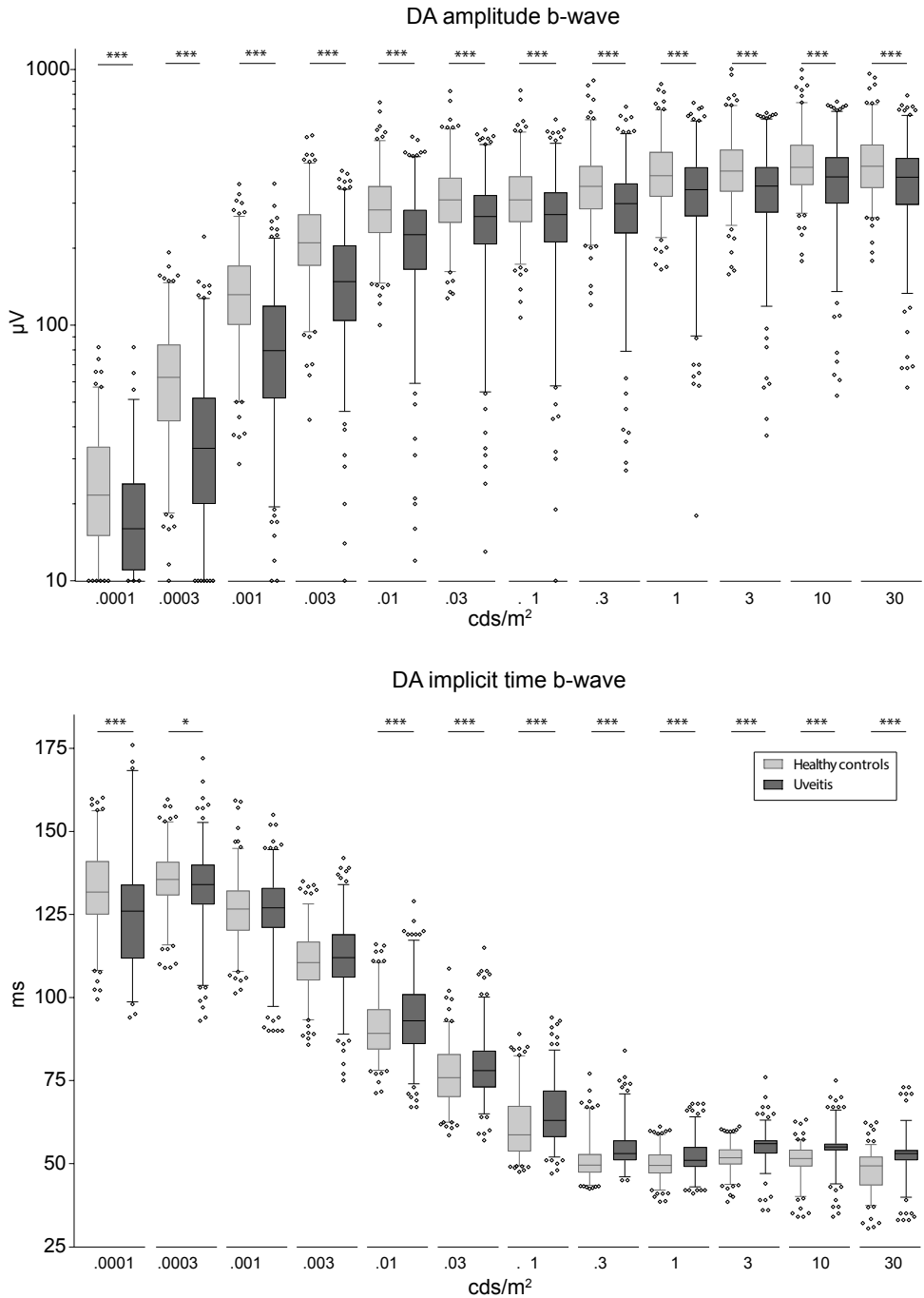
*Supplemental figure 2: Boxplots of the amplitudes and implicit times of different flash intensities of the single flash dark-adapted b-wave of uveitis patients compared to healthy controls. Statistical testing was performed using Mann-Whitney-U test. * P-value < 0.05. *** P-value of < 0.001. Abbreviations: cds/m² = candela · seconds/squared meters, DA = dark adapted, ms = milliseconds, μV = microvolts.*
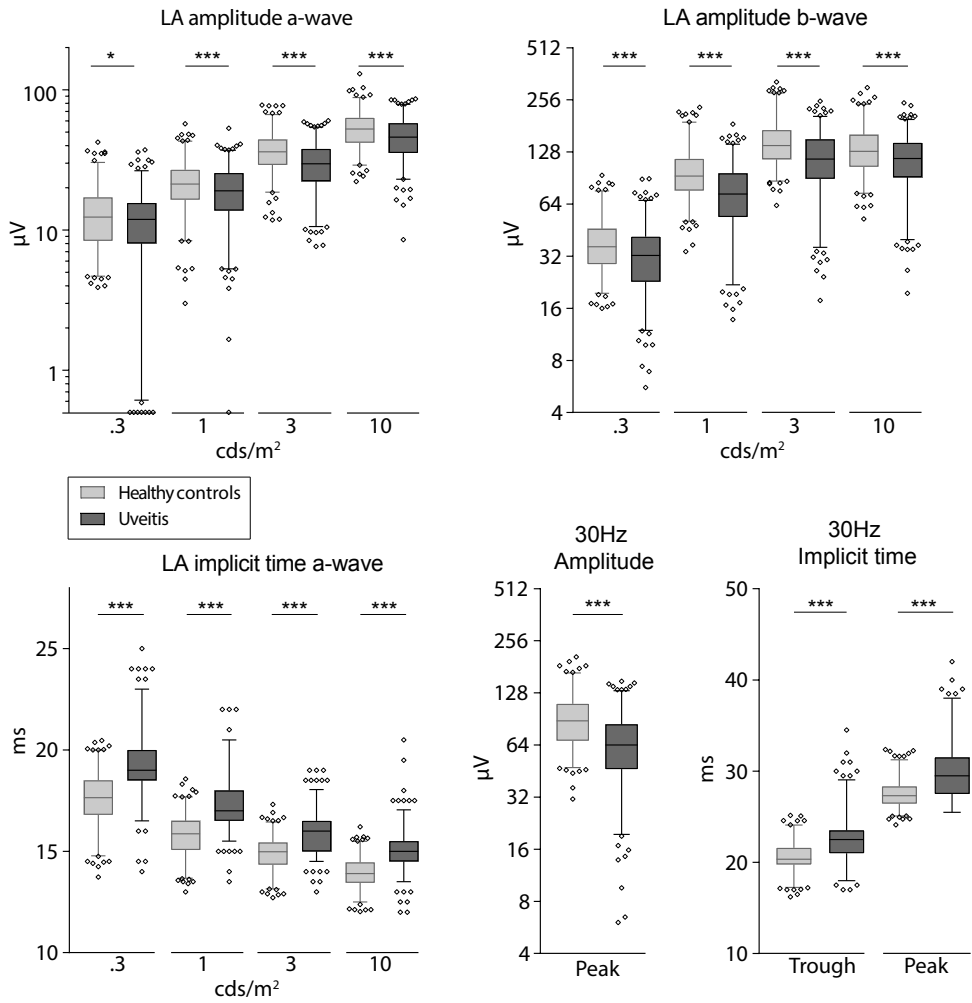
*Supplemental figure 3: Boxplots of the light adapted a-wave (implicit times and amplitudes). B-wave (amplitudes) of different flash intensities and the 30 Hz flicker response of uveitis patients compared to healthy controls. Statistical testing was performed using Mann-Whitney-U test. *** P-value of < 0.001. Abbreviations: cds/m² = candela · seconds/squared meters, LA = light adapted, ms = milliseconds, μV = microvolts.*
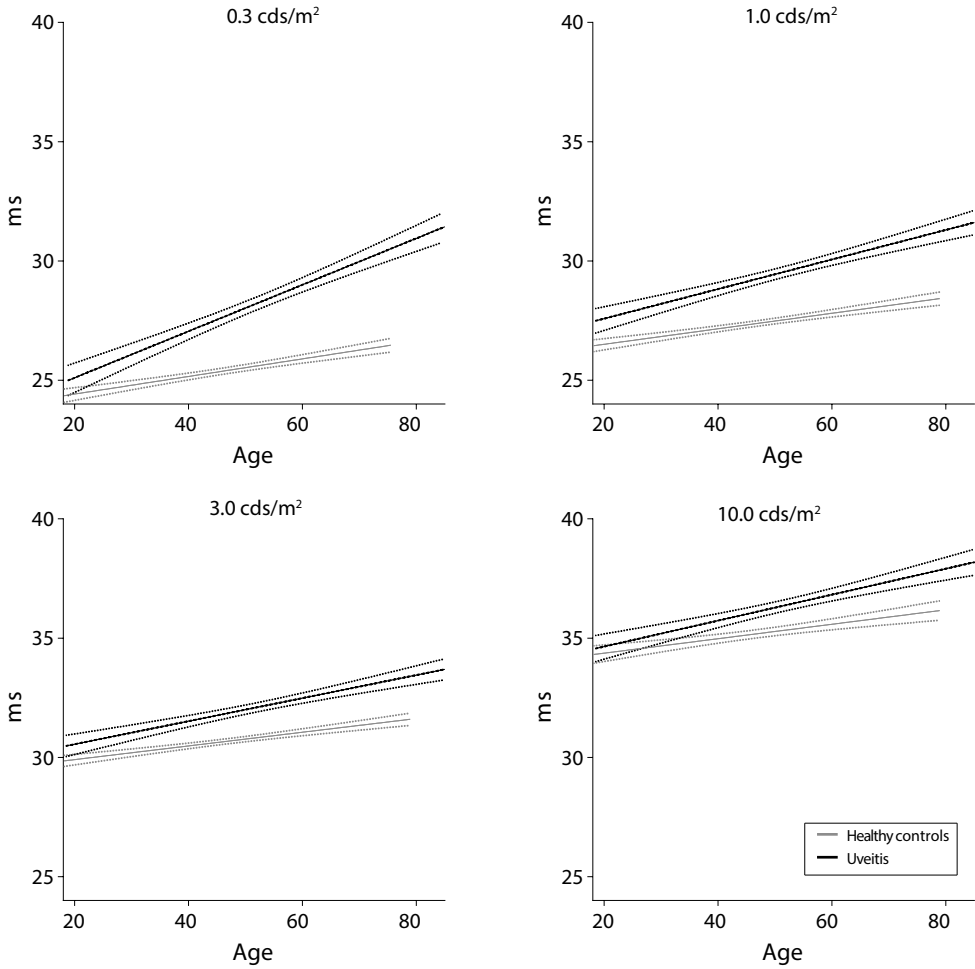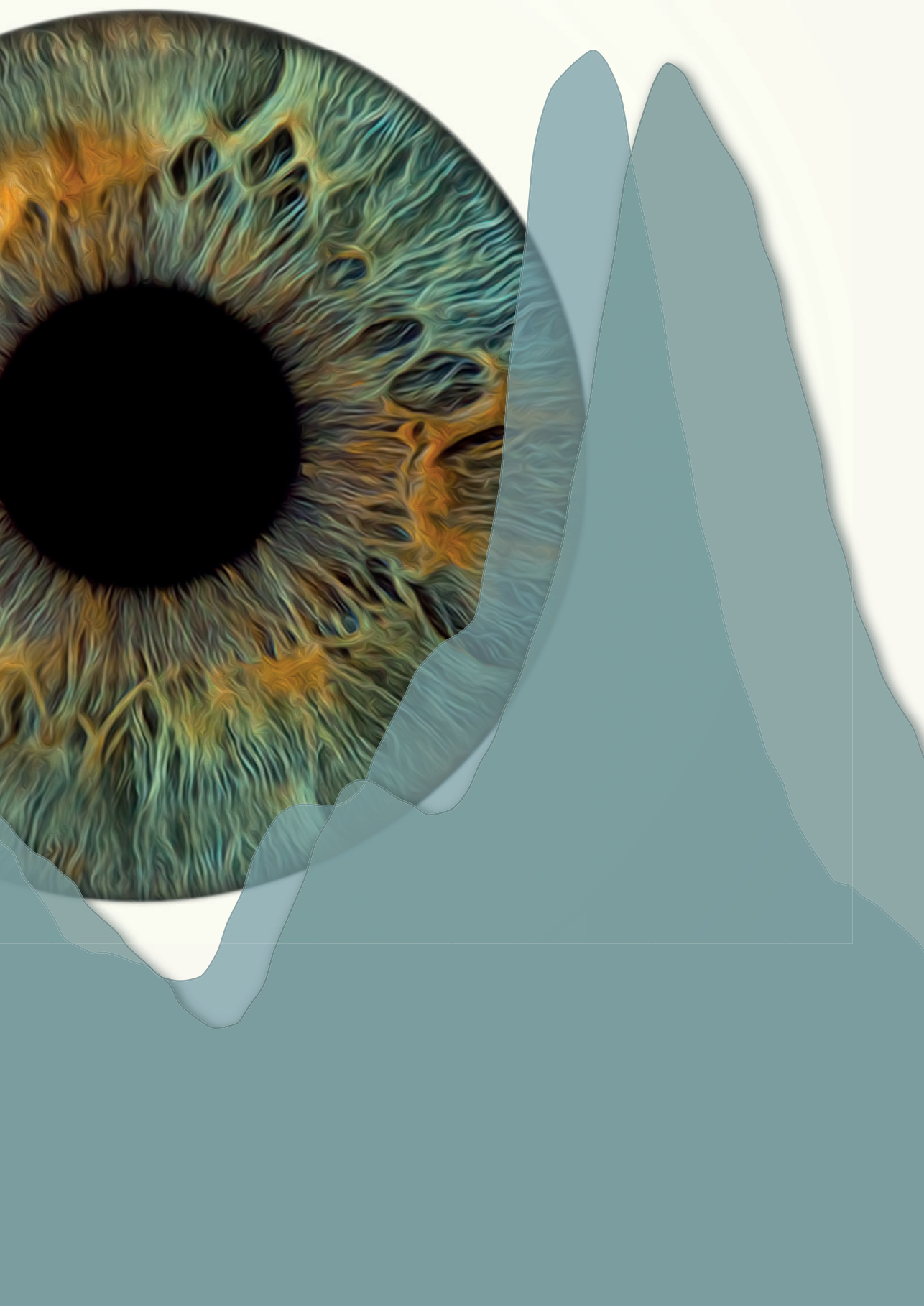
Supplemental figure 4: Logistic regression of the effect of age on the implicit time of the single flash cone b-wave (shown in ms on the y-axis) of uveitis patients compared to healthy controls. Abbreviations: cds/m² = candela · seconds/squared meters, ms = milliseconds.
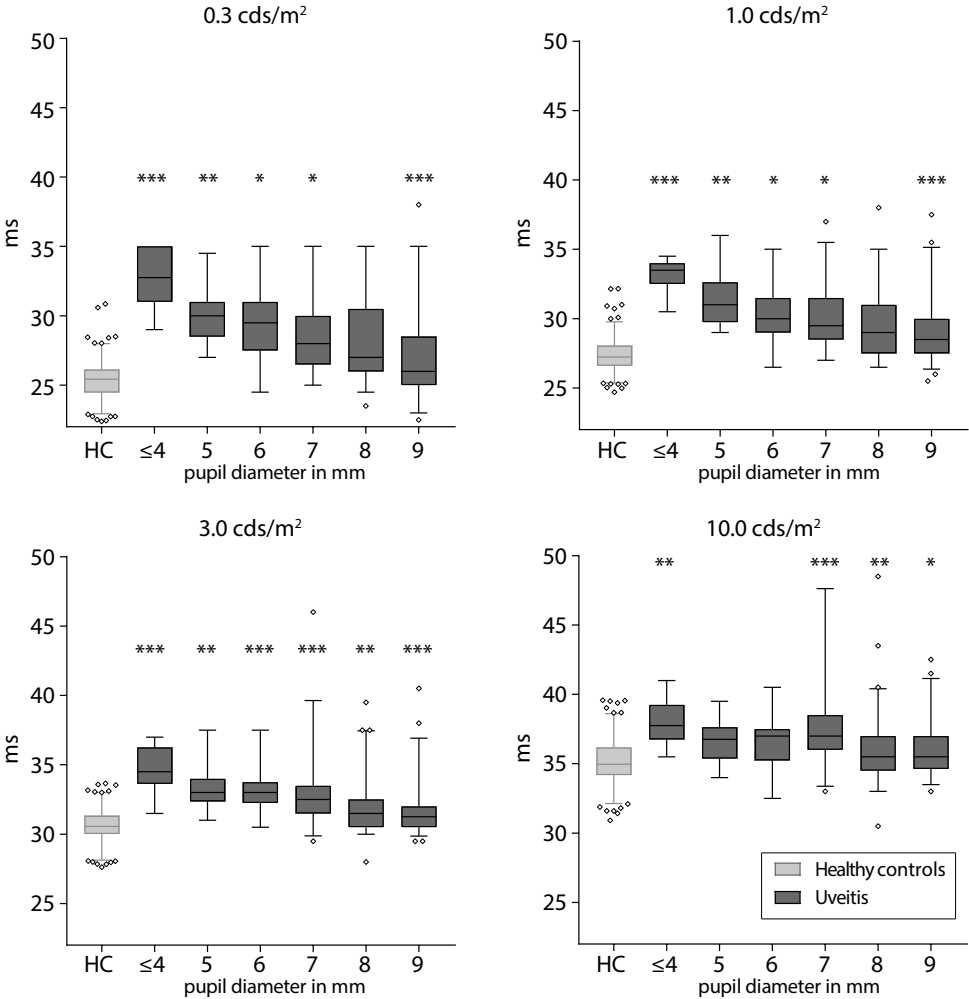
Supplemental figure 5: Effect of different pupil sizes on the implicit time of the single flash cone b-wave (shown in ms on the y-axis) of uveitis patients compared to healthy controls (HC) with fully dilated pupils. Statistical testing was performed using Mann-Whitney-U test. * P-value < 0.05. ** P-value < 0.01. *** P-value of < 0.001. Abbreviations: cds/m² = candela · seconds/squared meters, ms = milliseconds.

CHAPTER 3

ELECTRORETINOGRAM ABNORMALITIES IN NON-INFECTIOUS UVEITIS OFTEN PERSIST

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ABSTRACT

Purpose

In uveitis, a prolonged implicit time of the cone b-wave is a characteristic electroretinogram (ERG) abnormality. We investigated whether this can improve or deteriorate over time and which clinical factors are associated with change.

Methods

Prospective cohort study. Patients with a non-infectious uveitis were included. An ERG was measured in the first year of uveitis onset and a follow-up ERG one year later. Changes in the implicit time of the cone b-wave were investigated in relation to clinical parameters including the following: demographics, uveitis characteristics, treatment, best corrected visual acuity, optical coherence tomography parameters and fluorescein angiography scores.

Results

Of 98 eyes (63 patients), 40 showed a prolonged cone b-wave on the first ERG, which improved in 10 eyes. Eyes with an improved ERG more often had a panuveitis with initially a higher incidence of cells in the anterior chamber during the first ERG, which resolved at the time of their follow-up ERG. Five of the 58 eyes with a normal first ERG, had a deteriorated follow-up ERG. These eyes had more frequently an active uveitis at the time of the follow-up ERG. Of the 78 eyes with a stable cone b-wave, 16 had a quiescent inflammation during follow-up. There were no differences in age or treatment.

Conclusion

In most patients with non-infectious uveitis, ERG abnormalities appear to be irreversible, even when the inflammation becomes quiescent. However, some ERGs improved, which was associated with reduction in inflammation of the anterior chamber due to panuveitis. In contrast a worsened ERG was associated with a persistence of inflammation.

INTRODUCTION

Uveitis comprises of a group of multiple diseases with an inflammation of the uvea and its adjacent structures, including the retina.[1] If this inflammation is severe, it can be treated with immune-modulating drugs. However, despite intensive treatment strategies with these medicines, vision loss can still develop.[2] This vision loss can be the result of several complications of intraocular inflammation.

Well-known complications are macular oedema, cataract and glaucoma.[3] However, retinal atrophy and loss of retinal function may also occur. Recently, we described a characteristic type of ERG abnormality in uveitis: a prolonged cone b-wave, which was seen in all anatomic subtypes including anterior uveitis. This prolonged cone b-wave was associated with a more severe inflammation of both present and past.[4]

So far it is unknown to what extent ERG abnormalities in uveitis are reversible. We found that the prolonged cone b-wave could still be present in eyes in which inflammation had become inactive at the time of ERG recording. However, there are reports of ERG abnormalities that improve in uveitis. For instance, in birdshot chorioretinitis (BSCR) the ERG can normalize after treatment.[5,6] On the other hand, in Behçet disease, the ERG does not improve, even when the inflammation becomes quiet.[7] In other uveitis entities, such as in AMPPE, MEWDS and AZOOR, the ERG may either improve or deteriorate over time.[8–10]

To gain more insights into factors that may contribute to either an improvement, or a worsening of retinal function in uveitis patients, we investigated changes in the ERG over time in relation to clinical factors such as anatomical localization and diagnosis, severity of inflammation and treatment.

METHODS

Design and patient population

This is a one-year follow-up study of a prospective cohort study which describes the retinal function of uveitis patients by using the ERG.[4] Inclusion criteria for this follow-up study were as follows: a non-infectious uveitis and a disease duration of less than 1 year at the time of inclusion for the previous study. The results of the first ERGs were part of a larger cohort study which was previously described.[4] The diagnoses which were included are as follows: Behçet disease, birdshot, chorioretinitis, human leukocyte antigen (HLA)-B27 associated uveitis, Vogt–Koyanagi–Harada disease, sarcoidosis, and uveitis of unknown cause.

We included 72 patients (114 uveitis eyes) for this follow-up study. Of the initial 80 patients who had an ERG in the first year of onset of uveitis, four no longer wanted to participate, three were referred back to another hospital and one moved to another country.

All patients were ≥ 18 years of age, mentally competent, and gave informed consent to participate. Patients were seen at the University Medical Centre Utrecht, a tertiary referral centre for uveitis. Patients with juvenile idiopathic arthritis, diabetic retinopathy, retinal dystrophy, family history of retinal dystrophy, myopic degeneration, or severe media opacities were excluded. Ethical approval was requested and obtained from the Medical Ethical Research Committee of the University Medical Centre Utrecht. This study was conducted in compliance with the ethical principles of the Declaration of Helsinki.

ERG analysis

Electroretinograms were performed on the same day as an outpatient clinic visit when uveitis activity was assessed. Electroretinograms were measured according to an extended protocol, with more flash strengths than the standard International Society for Clinical Electrophysiology of Vision (ISCEV) protocol.[11] The flash strengths increase with approximately 0.5 log units steps and range from 0.0001 to 30.0 cds/m^2 (12 flash strengths) for the dark-adapted ERG (DA) and from 0.3 to10.0 cds/m^2 (4 flash strengths) for the light-adapted ERG (LA) and include a 30 Hz flicker response as well.

We used Dawson-Trick-Litzkow (DTL) electrodes as active electrodes, and an Espion E3 system with ColorDome stimulator (Diagnosys LLC, Cambridge, UK) for flash stimulation. Full details of the ERG measurement procedure, as well as our reference values, were previously described.[4]

In this study we focus on changes in the implicit time of the cone b-wave, but other aspects of the ERG were investigated as well. The implicit time of the cone b-wave is correlated to the peak implicit time of the 30 Hz flicker response (Spearman's rho coefficient 0.620 - 0.814 $p < .001$).[4] Because the prolonged cone b-wave was the most frequent and characteristic ERG abnormality in uveitis that we observed in our previous study, we focused on differences in the cone b-wave

To our knowledge there are no internationally accepted criteria to define whether an ERG has improved or worsened, but only criteria to describe whether an ERG is normal or abnormal (in- or outside reference values). In this study we used the difference in milliseconds (ms) of the implicit time of the cone b-wave of first ERG minus the follow-up ERG to define whether the ERG was stable, improved or worsened. Because pupil size affects the implicit time of the cone b wave, we excluded eyes with a pupil size difference > 1 mm between the first and follow-up ERG.

To determine how many ms the implicit time needed for change to be defined as significantly changed, we used data of a previous study where we measured a LA ERG twice in 200 uveitis patients, to assess the effects of DTL position on the ERG.[12] The DTL position had an effect on amplitudes, but not on implicit times of the ERG. Because this study was in essence a repeated measurement study, we used the difference in implicit time between the two ERGs of each eye to determine a normal repeatability distribution. We defined improvement as a reduction in cone b- wave implicit time of ≥ 2 SD of this distribution (2.7, 1.7, 1.6 and 1.3 ms for the 0.3, 1.0, 3.0 and 10.0 cds/m² flashes respectively) in at least two consecutive flash strengths. We defined worsening as an increase in implicit time with ≥ 2 SD in at least two consecutive flash strengths. We defined the ERG as stable if the implicit time of the first and second ERG was within 95% of the repeatability distribution (< 2 SD). Figure 1 shows examples of a patient with a worsened implicit time (A) and of a patient with an improved implicit time of the cone b-wave (B).

Because the focus of this manuscript is on the implicit time of the cone b-wave of the ERG, the terms stable, improved, or worsened ERG mean a stable, improved, or worsened implicit time of the cone b-wave, respectively.

Clinical parameters

Medical records were reviewed for age, gender, medical history, uveitis diagnosis, and anatomical localization of uveitis. On each of the two outpatient clinical visits we recorded for each eye the BCVA and graded uveitis activity according to the SUN criteria.[13] We also noted the presence of possible media opacities and other factors which might influence the ERG such as: posterior synechiae, corneal clarity, lens clarity, vitreous haze, and pupil size.

Also, we recorded per patient if they were treated at the time of ERG recording with systemic steroids, disease modifying anti-rheumatic drugs (DMARDS) (i.e. methotrexate (MTX), azathioprine, mycophenolate mofetil, mycophenolate sodium, or cyclosporine) or biologicals (adalimumab or infliximab).

We scored fluorescein angiograms (FA) which were performed within 3 months to the time of ERG recordings as an indication of the severity of inflammation. FAs were scored by an experienced ophthalmologist (JdB) who was blinded regarding the ERG results, using the Fluorescein angiographic scoring system of the Angiography Scoring for Uveitis Working Group (ASUWOG).[14] This FA scoring system scores individual aspects of an FA, which are added up to a final FA score. This FA score, which is the summation of each of these individual sub-scores helps to quantify the magnitude of retinal inflammation. In our previous study, eyes with a higher FA score

more frequently had a prolonged cone b-wave.[4] If FAs were performed on the same day as the ERG, there was at least an hour between the FA and ERG.[15]

To determine whether cystoid macula oedema (CMO) was present, we used optical coherence tomography (OCT) (Zeiss, Cirrus HD OCT 5000) scans of the same day as the ERG. We defined an active uveitis as the presence of cells in the anterior chamber and/or a vitritis of ≥ 1+ cells and/or a fluorescein angiograms (FA) score of > 1.

Statistical analysis

For statistical analysis R version 1.1.456 (© 2009 - 2018 RStudio, Inc.) was used. ERG changes between the first and the follow-up ERG were investigated using the paired t-test or Wilcoxon paired sample test, depending on the distribution of data. Data distribution was evaluated with qq-plots, histograms and Shapiro Wilk test. All significances were two tailed. We considered p-values < 0.05 as statistically significant.

Next, we investigated whether eyes that had a prolonged cone b-wave during the first ERG could improve, or worsen even more in the follow-up ERG. Also, we investigated whether eyes that had an implicit time that was within our reference values, could worsen in the follow-up ERG. These outcomes were then investigated in relation to clinical parameters using Pearson chi-square test or Fischer's exact test for categorical variables, and a Student t-test or a Mann-Whitney-U test for continuous variables, depending on the distribution of data.

For patient specific characteristics such as age, one eye per patient was analysed. For patients with a bilateral uveitis where both eyes had the same cone b-wave outcome (i.e. both uveitis eyes stable, improved, and worsened), one eye was selected at random. If the cone b-wave outcome differed between uveitis eyes of the same patients, the eye with the changed cone b-wave outcome (i.e. improved or worsened) was selected for analysis of patient-specific variables such as age or diagnosis. None of the patients had one eye which improved and one that worsened.

RESULTS

ERG results

Sixteen eyes of nine patients were excluded from further analysis, because the pupil size difference was > 1 mm between the two ERGs and a disparity in pupil size can cause a change in b-wave implicit time that is independent of retinal pathology. Of these excluded eyes, 2 eyes showed an improvement, 1 eye worsened and 13 eyes had no significant difference in the implicit time of the cone b-wave.

On a group level, the implicit times of the cone b-wave were not statistically significantly different between the first and the follow-up ERG (see Table 1). The ERG changed significantly in 20 eyes: in 10 eyes the implicit time improved and in 10 eyes it worsened, based on our cut-off values (see Figure 1 for examples).

There was no predominant flash strength at which this change in implicit time was most prominent. In other ERG parameters (see Supplemental table 1), there were also no consistent significant changes between the first and the follow-up ERG. There was a significant correlation between the peak implicit time of the 30Hz flicker response and a prolonged cone b-wave of both the first (Spearman's rho = 0.739, p < 0.001) and follow-up ERG (Spearman's rho = 0.695, p < 0.001)

Table 1: Results of implicit time of the cone b-wave of the first and follow-up electroretinogram

Implicit time cone b-wave, median [IQR]		1st ERG (< 1 year of diagnosis)	Follow-up ERG (< 1 year later)	P-value
cds/m²	0.3	27.00 [25.50, 30.50]	27.00 [26.00, 30.38]	0.544
	1.0	29.25 [28.00, 31.00]	29.25 [28.00, 31.00]	0.960
	3.0	31.50 [30.62, 33.50]	32.00 [31.00, 33.50]	0.760
	10.0	35.50 [35.00, 37.50]	35.75 [35.00, 37.00]	0.510

Results of the first and follow-up ERG of the different flash strengths (cds/m²) of the implicit time of the cone b-wave of 98 uveitis eyes. Eyes with a pupil size difference > 1 mm between the first and the follow-up ERG were excluded. Results of the other ERG parameters are shown in supplementary table 1. P-values were tested with the paired Wilcoxon signed rank test (no normal distribution of data). All significances were two-tailed. Abbreviations: ERG = electroretinogram, IQR = interquartile range, cds/m² = candela · seconds/squared meters.

ERG changes in relation to clinical parameters

Table 2 shows possible associations between changes in the ERG and patient characteristics as well as treatment. Regarding age, gender, anatomical localization, and uveitis diagnosis there were no differences in changes in the implicit time of the cone b-wave.

We also observed no statistically significant differences in treatment between patients with a different ERG outcome. However, more patients that had an improved follow-up ERG were treated with systemic medication after the first ERG. In contrast, one of the patients with a worsened ERG ceased systemic medication between the first and follow-up ERG.

There was no significant association between the duration of uveitis and start of systemic medication with either an improvement or a worsening of the ERG. However, patients with an abnormal first ERG, started systemic medication later compared to
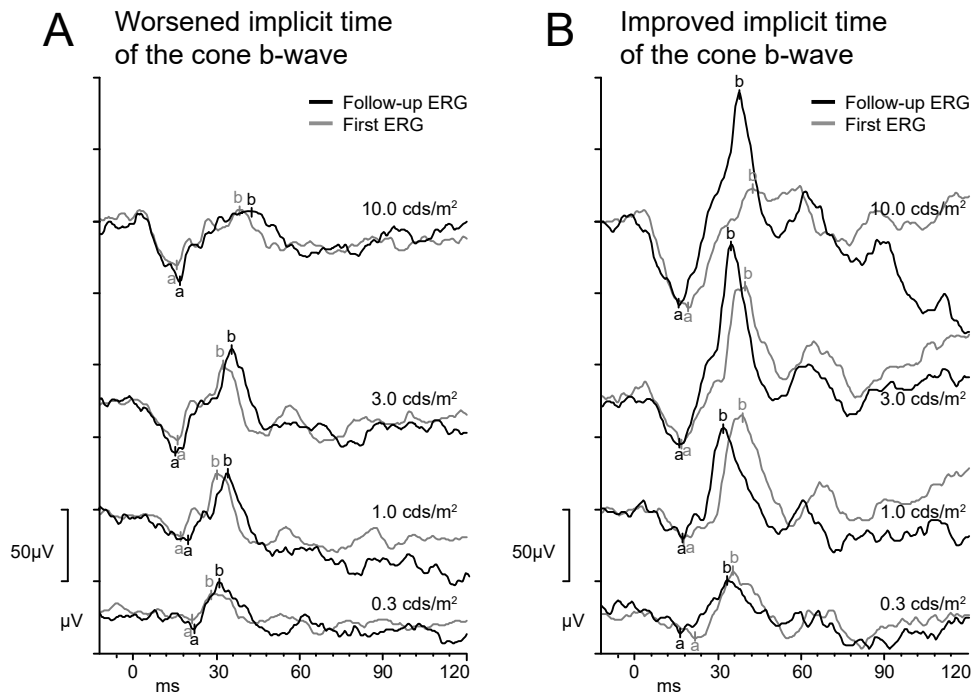
Figure 1: Examples of a patient with a worsened (A) implicit time of the cone b-wave, and of a patient with an improved implicit time of the cone b-wave (B). The first ERG is printed in grey and the follow-up ERG a year later in black.

patients with a normal first ERG (median uveitis duration on months of 1.2 versus 2.9, respectively, p = 0.382). This difference was not significant and overall patients with an abnormal first ERG were treated more often with systemic medication. Also, we observed no differences based on uveitis diagnosis.

Table 3 shows possible associations for an improved implicit time of the cone b-wave and uveitis characteristics. Eyes with an improved implicit time more often had a panuveitis with cells in the anterior chamber during the first ERG. This inflammation in the anterior chamber was resolved during the second ERG in all improved eyes.

Table 4 shows possible associations between a worsening of the implicit time of the cone b-wave in eyes with an initial normal ERG and uveitis characteristics. Eyes that had a worsened ERG, more frequently had an active uveitis during the second ERG. This activity was mostly due to a vitritis, which was often not present during the first ERG. Besides vitritis, BCVA of these eyes was on average also slightly worse during the first ERG, but improved during the follow-up ERG.

Eyes with an improved ERG had more frequently media opacities and/or a small pupil size during the first ERG, whereas eyes with a worsened ERG had these findings more frequently during the second ERG.

Table 2. Patient characteristics of uveitis patients with different outcomes of the implicit time of the cone b-wave

	Prolonged cone b-wave first ERG				Normal cone b-wave first ERG		
	Improved during FU ERG (n=8)	Stable during FU ERG (n=16)	Worsened during FU ERG (n=4)	p-value	Stable during FU ERG (n=30)	Worsened during FU ERG (n=5)	p-value
Age[a], median [IQR]	60.97 [54.40, 67.00]	59.21 [52.13, 67.91]	51.58 [44.92, 61.43]	0.694	35.10 [25.26, 52.26]	49.56 [40.45, 53.93]	0.239
Male gender, N (%)	6 (37.5)	5 (62.5)	1 (25.0)	0.492	11 (36.7)	3 (60.0)	0.369
Bilateral uveitis, N (%)	11 (68.8)	5 (62.5)	2 (50.0)	0.863	14 (46.7)	3 (60.0)	0.658
Localisation, N (%)				0.241			0.119
Anterior	0	1 (12.5)	0		1 (3.3)	0	
Intermediate	2 (12.5)	1 (12.5)	0		1 (3.3)	2 (40.0)	
Posterior	7 (43.8)	5 (62.5)	4 (100.0)		18 (60.0)	2 (40.0)	
Panuveitis	7 (43.8)	1 (12.5)	0		10 (33.3)	1 (20.0)	
Diagnosis, N (%)				0.364			0.555
Unknown cause	9 (56.2)	6 (75.0)	1 (25.0)		8 (26.7)	3 (60.0)	
Chorioretinitis	1 (6.2)	0 (0.0)	1 (25.0)		10 (33.3)	1 (20.0)	
Sarcoidosis	2 (12.5)	2 (25.0)	0		6 (20.0)	0	
Birdshot	2 (12.5)	0 (0.0)	2 (50.0)		2 (6.7)	1 (20.0)	
VKH	1 (6.2)	0	0		0	0	
Behcet	0	0	0		1 (3)	0	
Systemic med 1st ERG, N (%)	8 (50.0)	3 (37.5)	3 (75.0)	0.514	14 (46.7)	1 (20.0)	0.365
Systemic med FU ERG, N (%)	11 (68.8)	4 (50.0)	3 (75.0)	0.551	4.46 [6.76]	1.10 [1.42]	0.502
Time start systemic med, median [IQR][b]	5.57 (5.95)	4.93 (6.25)	2.86 (3.43)	0.777	1.73 [1.46, 1.92]	1.65 [1.40, 1.86]	0.576

In case of a bilateral uveitis, one eye per patient was analysed. When both uveitis eyes had the same cone b-wave outcome (i.e. both uveitis eyes stabile, or both improved, or both worsened), one eye was selected at random. If the cone b-wave outcome differed between uveitis eyes in patients with a bilateral uveitis, the eye with the changed cone b-wave outcome (i.e. improved or worsened) was selected for analysis of patient specific variables such as age or diagnosis. None of the patients had one eye with an improved ERG and one eye with a worsened ERG. a) In years. b) Time in months between the onset of uveitis and the start of systemic medication. Abbreviations: FU = follow-up, N = number, IQR = interquartile range, 1st = first, ERG = electroretinogram, med = medication.

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Table 3. Uveitis activity and vision of eyes (40) with a prolonged cone b-wave during their first electroretinogram

Uveitis activity and vision of eyes with a prolonged cone b-wave at their first ERG

	At time of first ERG[a]				At time of follow-up ERG[b]			
	Improved at time of FU ERG (n = 10)	Stable at time of FU ERG (n = 25)	Worsened at time of FU ERG (n = 5)	p-value	Improved at time of FU ERG (n = 10)	Stable at time of FU ERG (n = 25)	Worsened at time of FU ERG (n = 5)	p-value
Active, N (%)[c]	9 (90)	20 (80)	4 (80)	0.847	4 (40)	9 (36)	4 (80)	0.210
BCVA, median [IQR]	0.12 [0.01, 0.22]	0.15 [0.05, 0.40]	0.15 [0.10, 0.52]	0.598	0.36 [0.04, 0.52]	0.15 [0.00, 0.40]	0.22 [0.15, 0.52]	0.566
Flare, N (%)	1 (10)	0	0	0.375	0	2 (8)	1 (20)	0.464
Cells AC, N (%)	5 (50)	3 (12)	0	0.030	0	0	1 (20)	0.125
Anterior uveitis	1 (10)	0	0		0	0	0	
anuveitis	4 (40)	3 (12)	0		0	0	1 (20)	
Vitritis, N (%)	5 (50)	11 (44)	2 (40)	>.999	3 (30)	6 (24)	3 (60)	0.291
Anterior uveitis	1 (10)	0	0		0	0	0	
Intermediate uveitis	1 (10)	0	0		0	2 (8)	0	
Posterior uveitis	3 (30)	5 (20)	2 (40)		2 (20)	4 (16)	3 (60)	
Panuveitis	0	6 (24)	0		1 (10)	0	0	
CME on OCT, N(%)	4 (40)	8 (32)	2 (40)	0.894	0	5 (20)	2 (40)	0.155
Mean deviation VF, median [IQR]	-15.1 [-15.1, -15.1]	-3.7 [-6.2, -1.5]	-4.9 [-7.5, -4.2]	0.462	-2.1 [-2.4, -1.9]	-4.4 [-7.5, -2.8]	-7.5 [-7.7, -5.1]	0.212
FA score 3mnd, median [IQR]	12.5 [6.8, 17.3]	6.0 [3.8, 9.5]	1.5 [0.8, 2.3]	0.095	11.0 [7.8, 12.5]	2.0 [1.5, 6.0]	20.0 [10.5, 20.0]	0.424
Media opacities and/or small pupil size N(%)[d]	8 (80)	5 (20)	0	<0.001	6 (24.0)	5 (63)	5 (100)	0.001

Clinical characteristic during the first and FU ERG of eyes with a prolonged cone b-wave during their first ERG. Eyes with a pupil size difference >1 mm between the first and the follow-up ERG were excluded. Statistical testing was done with Fischer's exact test in case of categorical variables and Mann-Whitney-U test in case of continuous variables. All tests were two-tailed. Data are presented as N (%), unless otherwise specified. a) < first year of uveitis activity. b) > 1 year later. c) Defined as the presence of cells in the AC and/or a vitritis of ≥1+ cells and/or a FA score of >1. d) Defined as opacities of the lens/IOL and/or vitreous opacities and/or a pupil size of < 6 mm. Abbreviations: N = number, IQR = interquartile range, AC = anterior chamber, CMO = cystoid macula oedema, OCT = optical coherence tomography, VF = visual field, FA = fluorescein angiography, FU = follow-up, IOL = intraocular lens, ERG = electroretinogram.

Table 4. Uveitis activity and vision of eyes (58) with a normal implicit time of the cone b-wave during their first electroretinogram

Uveitis activity and vision

Clinical characteristic	At time of first ERG[a]			At time of follow-up ERG[b]		
	Stable during FU ERG (n = 53)	Worsened during FU ERG (n = 5)	p-value	Stable during FU ERG (n = 53)	Worsened during FU ERG (n = 5)	p-value
Active, N (%)[c]	22 (44)	2 (40)	>.999	7 (13.7)	3 (60)	0.035
BCVA, median [IQR]	0.00 [-0.08, 0.05]	0.05 [0.05, 0.10]	0.120	-0.05 [-0.08, 0.05]	0.00 [0.0, 0.00]	0.531
Flare, N (%)	1 (2)	0	>.999	2 (4)	0	>.999
Cells AC, N (%)	5 (10)	0	>.999	1 (2)	0	>.999
Anterior uveitis		0		0	0	
Panuveitis		0		1 (2)	0	
Vitritis, N (%)	9 (18)	2 (40)	0.259	3 (6)	2 (40)	0.058
Anterior uveitis	0	0		0	0	
Intermediate uveitis	2 (4)	2 (40)		0	1 (20)	
Posterior uveitis	2 (4)	0		3 (6)	0	
Panuveitis	5 (9)	0		0	1 (20)	
CME on OCT, N(%)	6 (12)	0	>.999	1 (2)	1 (20)	0.166
Mean deviation VF, median [IQR]	-1.3 [-2.8, -0.3]	-1.6 [-1.8, -1.3]	0.802	-1.5 [-2.5, -0.4]	-1.1 [-1.4, -0.8]	0.683
FA score 3mnd, median [IQR]	1.5 [0.0, 4.5]	1.0 [0.0, 2.5]	0.654	0.0 [0.0, 1.5]	0.0 [0.0, 0.0]	0.568
Media opacities and/or small pupil size N(%)[d]	7 (14)	2 (40)	0.184	12 (25)	1 (20)	>.999

Clinical characteristic during the first and FU ERG of eyes with a normal implicit time of the cone b-wave during their first ERG. Eyes with a pupil size difference > 1 mm between the first and the follow-up ERG were excluded. Statistical testing was done with Fischer's exact test in case of categorical variables and Mann-Whitney-U test in case of continuous variables. All tests were two-tailed. Data are presented as N (%), unless otherwise specified. a) < first year of uveitis activity. b) > 1 year later. c) Defined as the presence of cells in the AC and/or a vitritis of ≥ 1+ cells and/or a FA score of >1. d) Defined as opacities of the lens/IOL and/or vitreous opacities and/or a pupil size of <6mm. Abbreviations: N = number, IQR = interquartile range, AC = anterior chamber, CMO = cystoid macula oedema, OCT = optical coherence tomography, VF = visual field, FA = fluorescein angiography, FU = follow-up, IOL = intraocular lens, ERG = electroretinogram.

It is important to note that most of the eyes did not show significant ERG changes. In 78 eyes (80%) the implicit time was stable, of which 25 eyes had a prolonged implicit time. Almost two third of these (16 eyes) had no signs of an active inflammation during the follow-up ERG.

Supplemental tables 2 and 3 give an overview of the different aspects of the FA score. There were no differences in these individually scored aspects of the FA between eyes with a different ERG outcome.

Supplemental table 4 shows the correlation matrix between statistically significant clinical factors, duration of uveitis, age, and treatment. Although many factors are statistically significant, all of the associations are weak (rho < 0.5).

DISCUSSION

This study highlights that a prolongation of the implicit time of the cone b-wave occurs early on in the non-infectious uveitis and often persists. Only in a minority of cases, it can improve or deteriorate in the course of the disease. Improvement was mostly seen in eyes in which the inflammation became more quiescent, whereas a worsening was seen in eyes with a persistent inflammation. The delayed cone b-wave is probably due to an impaired photo-transmission to the bipolar cells. It remains speculative if this ERG abnormality is the first sign of evolving photoreceptor damage.

Although these findings indicate that it is important to treat the inflammation in uveitis adequately and early, we found no differences between the groups based on type of treatment. This might be due to several factors including variation between treatment strategies, as well as the effects of responders versus non-responders.

In eyes with an improved ERG, the inflammation in the anterior chamber had become quiescent. The majority of these patients had a panuveitis. It is likely that the improvement of the inflammation in the anterior chamber was associated with an improvement of the posterior segment, which should be the subject of further studies.

In most eyes the ERG abnormalities were irreversible, even without signs of active inflammation at the time of the follow-up ERG. Because we measured the first ERG in the first year of onset of disease, it indicates that the retinal damage can occur early on in the disease process. Patients with an abnormal first ERG started systemic treatment on average later than those with a normal first ERG. Furthermore, patients with an improved ERG were using systemic medication more often at the time of the follow-up ERG compared to the first ERG. Although these results showed a trend, the differences were not significant. One explanation could be that the patients in this study may represent a group with a more severe disease course, because this study was performed in a tertiary referral centre.

Apart from the severity of inflammation, eyes with an improved ERG had more frequently media opacities or a small pupil size during their first ERG. In the light-adapted ERG implicit times increase when the intensity of the flash strength becomes stronger. Media opacities or a small pupil size can reduce the retinal illuminance of the stimulus flash leading to changes in b-wave implicit time of non-pathologic origin.[4,12] Therefore, it is possible that the improved implicit time of the cone b-wave may even be underestimated due to the improvement of media opacities. Effects of changes in pupil size were addressed by excluding eyes with a pupil size difference > 1 mm.

Because the implicit time of the cone b-wave in uveitis over time has not previously been studied, it is not possible to make comparisons with literature. However, there are some reports of follow-up ERG's in birdshot uveitis that show that the 30 Hz flicker response can improve after systemic treatment with corticosteroids, but not in all cases.[5] Furthermore, other ERG parameters than the 30 Hz flicker response can be permanently affected in birdshot uveitis.[16,17]

In the current study, we could only replicate that the 30 Hz flicker response improved in the minority of the birdshot patients after the start of systemic treatment, possibly because 6 out of 7 of the included birdshot patients already used systemic medication at the time of the first ERG. In these patients the implicit time of the 30 Hz flicker response did improve in 4 out of 14 eyes, whereas the implicit time of the cone b-wave did not improve.

Some studies state that a change of > 30% in amplitude is necessary to define improvement or deterioration, based on Berson's study on decline in retinitis pigmentosa (RP).[18] However, the ERG in RP is generally much more severely affected than in uveitis, where the amplitude often does not change significantly. Most studies that report on follow-up results of the ERG in uveitis, do not specify how improvement is defined,[5,7–10] for instance, which aspect of the ERG changed during follow-up. In our study we observed that the different parameters of the ERG may behave quite differently. For uveitis monitoring, we found that the implicit time of the cone b-wave is the most sensitive parameter.

In summary, this study demonstrates that a prolonged implicit time of the cone b-wave often persists in uveitis. In a minority of cases, this loss of retinal function can improve if the inflammation becomes less active. This emphasizes the importance to treat the inflammation adequately and early in uveitis. Further research is needed to investigate to which extent the implicit time of the cone b-wave can be used as a prognostic marker in uveitis.

ACKNOWLEDGEMENTS

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SUPPLEMENTAL MATERIAL

Supplemental table 1: ERG results of the 1st and follow-up ERG (continued on next page)

			1st ERG (<1 year of diagnosis)	Follow-up ERG (<1 year later)	P-value
Light adapted	A-wave amplitude, mean (SD)				
	cds/m²	0.3	-12.52 (6.77)	-12.56 (6.58)	0,717
		1.0	-19.80 (8.73)	-19.14 (7.82)	0,201
		3.0	-30.35 (12.21)	-29.81 (11.07)	0,568
		10.0	-47.26 (16.10)	-46.01 (15.43)	0,125
	A-wave implicit time, median [IQR]				
	cds/m²	0.3	19.00 [18.50, 20.50]	19.00 [18.50, 20.00]	0,984
		1.0	17.00 [16.50, 18.00]	17.50 [16.50, 18.00]	0,502
		3.0	15.50 [15.00, 16.50]	16.00 [15.50, 16.50]	0,261
		10.0	15.00 [14.50, 15.50]	15.00 [14.50, 15.50]	0,990
	B-wave amplitude, mean (SD)				
	cds/m²	0.3	34.18 (15.86)	32.28 (13.58)	0,128
		1.0	76.00 (34.13)	72.81 (30.38)	0,165
		3.0	121.46 (48.46)	118.22 (45.11)	0,215
		10.0	114.65 (41.47)	115.13 (39.57)	0,695
	B-wave implicit time, median [IQR]				
	cds/m²	0.3	27.00 [25.50, 30.50]	27.00 [26.00, 30.38]	0,703
		1.0	29.25 [28.00, 31.00]	29.25 [28.00, 31.00]	0,762
		3.0	31.50 [30.62, 33.50]	32.00 [31.00, 33.50]	0,410
		10.0	35.50 [35.00, 37.50]	35.75 [35.00, 37.00]	0,326
	30 Hz				
		Peak amplitude, mean (SD)	67.31 (27.52)	68.18 (24.94)	0,463
		Trough implicit time, median [IQR]	22.00 [20.50, 23.50]	21.50 [20.50, 23.00]	0,819
		Peak implicit time, median [IQR]	29.50 [27.50, 31.88]	29.00 [27.00, 31.00]	0,275
Dark adapted	A-wave amplitude, median [IQR]				
	cds/m²	0.0001	-1.73 [-6.88, 1.18]	-4.24 [-12.57, 0.71]	0,454
		0.0003	-1.18 [-7.42, 4.69]	0.23 [-5.13, 3.68]	0,819
		0.001	-1.71 [-7.60, 3.21]	-2.06 [-8.64, 1.63]	0,579
		0.003	-6.39 [-13.36, -0.52]	-4.65 [-10.33, 2.37]	0,082
		0.01	-14.36 [-26.21, -7.36]	-13.05 [-22.74, -3.65]	0,043
		0.03	-30.32 [-48.85, -17.49]	-28.52 [-42.28, -13.20]	0,047
		0.1	-46.32 [-67.79, -34.05]	-44.00 [-59.88, -27.61]	0,146
		0.3	-92.76 [-136.25, -58.92]	-80.69 [-111.45, -56.38]	0,021
		1.0	-172.60 [-215.60, -110.63]	-152.45 [-209.88, -113.97]	0,179
		3.0	-192.30 [-254.08, -151.47]	-176.85 [-233.13, -140.60]	0,052
		10.0	-251.55 [-307.05, -185.10]	-230.85 [-280.22, -190.55]	0,047
		30.0	-257.95 [-324.75, -192.35]	-238.00 [-289.03, -198.20]	0,053

Supplemental table 1 (continued)

		1st ERG (<1 year of diagnosis)	Follow-up ERG (<1 year later)	P-value
A-wave implicit time, median [IQR]				
cds/m²	0.0001	80.50 [75.00, 83.00]	77.50 [73.00, 82.50]	0,233
	0.0003	76.00 [71.50, 80.00]	74.00 [71.00, 77.88]	0,041
	0.001	62.75 [60.00, 66.00]	62.00 [59.50, 65.50]	0,080
	0.003	51.50 [48.50, 55.00]	51.50 [48.00, 54.88]	0,946
	0.01	41.00 [38.50, 43.62]	41.50 [39.50, 43.50]	0,308
	0.03	34.00 [32.50, 37.00]	34.50 [33.00, 36.50]	0,089
	0.1	29.50 [28.50, 32.00]	29.50 [28.50, 31.50]	0,729
	0.3	27.00 [26.00, 28.88]	27.50 [26.50, 28.50]	0,801
	1.0	24.50 [24.00, 26.00]	25.00 [24.00, 25.50]	0,269
	3.0	17.00 [16.50, 18.50]	17.00 [16.50, 18.00]	0,664
	10.0	15.00 [13.12, 16.00]	14.50 [13.50, 16.00]	0,847
	30.0	12.50 [11.50, 14.00]	12.50 [11.50, 14.00]	0,297
B-wave amplitude, median [IQR]				
cds/m²	0.0001	18.16 [11.65, 28.13]	20.29 [12.49, 26.34]	0,824
	0.0003	31.64 [19.55, 56.86]	35.05 [21.91, 56.88]	0,895
	0.001	83.87 [51.51, 128.62]	91.31 [60.25, 123.40]	0,421
	0.003	146.00 [100.00, 214.10]	160.90 [106.25, 204.95]	0,618
	0.01	221.80 [160.80, 300.30]	231.15 [170.18, 285.07]	0,772
	0.03	254.85 [186.20, 337.50]	271.45 [201.42, 332.65]	0,524
	0.1	266.80 [198.43, 328.05]	272.95 [203.35, 329.10]	0,571
	0.3	286.60 [225.35, 368.78]	292.30 [229.40, 357.28]	0,988
	1.0	338.40 [261.20, 431.77]	334.95 [277.52, 400.07]	0,763
	3.0	344.40 [265.22, 420.30]	343.50 [277.72, 428.45]	0,658
	10.0	379.55 [293.97, 453.48]	377.90 [304.97, 443.70]	0,977
	30.0	381.00 [288.85, 452.47]	370.75 [298.27, 433.52]	0,615
B-wave implicit time, median [IQR]				
cds/m²	0.0001	127.25 [111.25, 136.50]	122.25 [114.50, 128.12]	0,745
	0.0003	135.25 [127.50, 142.25]	134.50 [130.00, 138.12]	0,336
	0.001	125.00 [121.00, 131.12]	126.50 [122.50, 132.50]	0,040
	0.003	110.00 [105.00, 116.00]	112.00 [106.12, 118.50]	0,254
	0.01	93.00 [85.50, 100.00]	91.50 [84.62, 99.25]	0,426
	0.03	77.00 [73.00, 82.88]	77.00 [73.62, 82.00]	0,368
	0.1	62.25 [57.00, 69.75]	65.25 [58.50, 71.50]	0,004
	0.3	52.50 [49.50, 57.50]	52.75 [50.62, 57.38]	0,103
	1.0	51.50 [48.50, 56.00]	51.75 [48.50, 55.50]	0,957
	3.0	55.00 [52.50, 57.00]	56.00 [52.50, 57.50]	0,094
	10.0	54.50 [53.50, 56.00]	54.25 [53.00, 55.88]	0,422
	30.0	52.50 [51.12, 54.00]	52.50 [51.50, 53.50]	0,381

(left margin label: Dark adapted)

Results of the first and follow-up ERG of the different flash strengths (cds/m²) and adaptation conditions of uveitis patients. Eyes with a pupil size difference >1mm between the first and the follow-up ERG were excluded. P-values were tested with the paired t-test or paired Wilcoxon signed rank test depending on the distribution of data. All significances were two-tailed. Abbreviations: ERG = electroretinogram, SD = standard deviation, IQR = interquartile range, cds/m² = candela · seconds/squared meters.

Supplemental table 2: Fluorescein angiography scores of eyes with a prolonged implicit time of the cone b-wave during their first ERG (continued on next page)

	At time of first ERG				At time of follow-up ERG			
	Improved at time of FU ERG (n =4)	Stable at time of FU ERG (n = 12)	Worsened at time of FU ERG (n =2)	p-value	Improved at time of FU ERG (n =4)	Stable at time of FU ERG (n = 3)	Worsened at time of FU ERG (n = 3)	p-value
FA score, median [IQR]	7.50 [5.50, 8.25]	6.00 [3.75, 11.25]	3.00 [3.00, 3.00]	0.446	11.00 [7.75, 12.50]	2.00 [1.50, 6.00]	20.00 [10.50, 20.00]	0.424
Optic disc, N(%)				0.597				0.050
0	1 (25)	3 (25)	1 (50)		1 (25)	2 (67)	0	
1	1 (25)	7 (58)	1 (50)		0	1 (33)	1 (33)	
2	0	1 (8)	0		3 (75)	0	0	
3	2 (50)	1 (8)	0		0	0	2 (67)	
Cystoid macula edema, N(%)				0.254				0.257
0	0	5 (42)	1 (50)		1 (25)	1 (33)	3 (100)	
1	2 (50)	1 (8)	1 (50)		0	1 (33)	0	
2	0	1 (8)	0		1 (25)	0	0	
3					0	1 (33)	0	
4	2 (50)	5 (42)	0 (0)		2 (50)	0	0	
Vasculitis posterior pole, N(%)				0.766				0.571
0	3 (75)	6 (50)	1 (50)		3 (75)	2 (67)	1 (33)	
1	0	1 (8)	1 (50)		1 (25)	0	0	
2	1 (25)	3 (25)	0		0	1 (33)	2 (67)	
3	0	2 (17)	0		0	0	0	
Vasculitis quadrants, N(%)				>.999				0.829
0	3 (75)	5 (42)	2 (100)		1 (25)	0	1 (33)	
1	0	1 (8)	0		1 (25)	2 (66.7)	0	
2	0	2 (17)	0		1 (25)	0	0	
3	0	1 (8)	0		0	0	0	
4	1 (25)	3 (25)	0		1 (25)	1 (33)	2 (67)	
Capp. leakage posterior pole, N(%)				0.652				0.371
0	3 (75)	8 (67)	1 (50)		1 (25)	3 (100)	1 (33)	
1	0	3 (25)	1 (50)		1 (25)	0	0	
2	1 (25)	1 (8)	0		2 (50)	0 (0)	2 (67)	
Focal capp. leakage quadrants, N(%)				>.999				0.167
0	4 (100)	10 (83)	2 (100)		1 (25)	3 (100)	3 (100)	
1	0	0	0		1 (25)	0	0	
2	0	1 (8)	0		2 (50)	0	0	
3	0	1 (8)	0		0	0	0	

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Supplemental table 2 (continued)

	At time of first ERG				At time of follow-up ERG			
	Improved at time of FU ERG (n =4)	Stable at time of FU ERG (n = 12)	Worsened at time of FU ERG (n =2)	p-value	Improved at time of FU ERG (n =4)	Stable at time of FU ERG (n = 3)	Worsened at time of FU ERG (n = 3)	p-value
Diffuse capp. leakage quadrants, N(%)				>.999				0.133
0	4 (100)	11 (92)	2 (100)		4 (100)	3 (100)	1 (33)	
4	0	0	0		0	0	0	
8	0	1 (8)	0		0	0	2 (67)	
non perfusion macula, N(%)				NA				>.999
0	4 (100)	12 (100)	2 (100)		3 (75)	3 (100)	3 (100)	
1	0	0	0		1 (25)	0	0	
non perfusion posterior pole, N(%)				NA				0.133
0	4 (100)	12 (100)	2 (100)		4 (100)	3 (100)	1 (33)	
1	0	0	0		0	0	2 (67)	
non perfusion quadrants, N(%)								>.999
0	4 (100)	11 (92)	2 (100)	>.999	3 (75)	3 (100)	1 (33)	
2	0	1 (8)	0		1 (25)	0	0 (0)	
neovascularisation elsewhere, N(%)				>.999				NA
0	4 (100)	11 (92)	2 (100)		4 (100)	12 (100)	2 (100)	
2	0	1 (8)	0		0	0	0	
pinpoint leakage, N(%)				0.111				NA
0	4 (100)	12 (100)	1 (50)		4 (100)	12 (100)	2 (100)	
2	0	0	1 (50)		0	0	0	

FAs of uveitis patients with a prolonged implicit time of the cone b-wave during their first ERG were scored using the Fluorescein angiographic scoring system of the Angiography Scoring for Uveitis Working Group (ASUWOG). Differences in FA score were compared between eyes with a stable, improved, or worsened implicit time of the cone b-wave. FAs were performed up to 3 months around the ERG. The first ERG was performed in the first year of inflammation, the follow-up ERG one year later. FAs with the highest overall FA score until the ERG was recorded are shown. Parts of the FA score were no leakage was visible (neovascularization optic disc and pooling) are not shown. Eyes with a pupil size difference >1mm between the first and the follow-up ERG were excluded. Abbreviations: FA = Fluorescein angiogram, N = number, IQR = interquartile range, capp. = capillary.

Supplemental table 3 (right): FAs of uveitis patients with a normal implicit time of the cone b-wave during their first ERG were scored using the Fluorescein angiographic scoring system of the Angiography Scoring for Uveitis Working Group (ASUWOG). Differences in FA score were compared between eyes with a stable or worsened implicit time of the cone b-wave. FAs were performed up to 3 months around the ERG. The first ERG was performed in the first year of inflammation, the follow-up ERG one year later. FAs with the highest overall FA score until the ERG was recorded are shown. Parts of the FA score were no leakage was visible (neovascularization optic disc , neovascularization elsewhere, pinpoint leakage, non-perfusion macula, non-perfusion posterior pole, and pooling) are not shown. Eyes with a pupil size difference >1mm between the first and the follow-up ERG were excluded. Abbreviations: ERG = electroretinogram, FA = Fluorescein angiogram, N = number, IQR = interquartile range, capp. = capillary.

Supplemental table 3: Fluorescein angiographic scores of eyes with a normal implicit time of the cone b-wave during their first ERG

	At time of first ERG			At time of follow-up ERG		
	Stable at time of FU ERG (n = 18)	Worsened at time of FU ERG (n =4)	p-value	Stable at time of FU ERG (n=11)	Worsened at time of FU ERG (n=1)	p-value
FA score, median [IQR]	4.00 [0.00, 6.75]	1.00 [0.00, 2.50]	0.205	0.00 [0.00, 1.50]	0.00 [0.00, 0.00]	0.568
Optic disc, N(%)			0.852			>.999
0	7 (39)	3 (75)		9 (82)	1 (100)	
1	4 (22)	0 (0)		1 (9)	0	
2	5 (28)	1 (25)		1 (9)	0	
3	2 (11)	0 (0)				
Cystoid macula edema, N(%)			>.999			>.999
0	13 (72)	4 (100)		10	1 (100)	
1	3 (17)	0 (0)		0	0	
2	0	0		1 (9)	0	
3	1 (6)	0 (0)		0	0	
4	1 (6)	0 (0)		0	0	
Vasculitis posterior pole, N(%)			>.999			>.999
0	11 (61)	4 (100)		10	1 (100)	
1	2 (11)	0 (0)			0	
2	3 (17)	0 (0)		1 (9)	0	
3	2 (11)	0 (0)			0	
Vasculitis quadrants, N(%)			0.419			>.999
0	11 (61)	2 (50)		8 (73)	1 (100)	
1	1 (6)	0 (0)		1 (9)	0	
2	2 (11)	2 (50)		1 (9)	0	
3	2 (11)	0 (0)		0	0	
4	2 (11)	0 (0)		1 (9)	0	
Capp. leakage posterior pole, N(%)			>.999			>.999
0	17	4 (100)			1 (100)	
1	1 (6)	0 (0)		1 (9)	0 (0)	
Focal capp. leakage quadrants, N(%)			>.999			NA
0		4 (100)		11 (100)	1 (100)	
4	1 (6)	0 (0)		0	0	
Diffuse capp. leakage quadrants, N(%)			>.999			NA
0		4 (100)		11 (100)	1 (100)	
4	2 (11)	0 (0)		0	0	
non perfusion quadrants, N(%)			NA			
0	18 (100)	4 (100)		10	1 (100)	>.999
2				1 (9)	0 (0)	
FAGNVOpD = 0 (%)	18 (100)	4 (100)	NA			
FAGNVE = 0 (%)	18 (100)	4 (100)	NA			
FAGpinL = 0 (%)	18 (100)	4 (100)	NA			
FAGpool = 0 (%)	18 (100)	4 (100)	NA			
FAGcapNPMac = 0 (%)	18 (100)	4 (100)	NA	11 (100)	1 (100)	NA
FAGcapNPPP = 0 (%)	18 (100)	4 (100)	NA	11 (100)	1 (100)	NA

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Supplemental table 4: Correlation matrix of significant clinical factors, as well as systemic medication, duration of uveitis, and age

		Cells AC 1st ERG	Active FU ERG[a]	Media opac./pupil <6mm 1st ERG[b]	Media opac./pupil <6mm FU ERG[b]	Age	Duration of uveitis 1st ERG	Duration of uveitis FU ERG	Syst. Med 1st ERG	Syst. Med FU ERG	Improved IT	Worsened IT
Cells AC 1st ERG	rho	1,000	0,084	0,144	0,176	0,031	,286**	0,179	-0,079	0,194	,362**	-0,137
	p-value		0,425	0,163	0,099	0,768	0,005	0,086	0,445	0,060	0,000	0,187
Active FU ERG[a]	rho	0,084	1,000	0,146	,354**	,258*	0,055	0,049	0,103	0,149	0,090	,318**
	p-value	0,425		0,164	0,001	0,011	0,595	0,636	0,319	0,147	0,383	0,002
Media opac./pupil <6mm 1st ERG[b]	rho	0,144	0,146	1,000	,257*	,337**	0,006	-0,050	-0,182	0,021	,462**	-0,026
	p-value	0,163	0,164		0,015	0,001	0,954	0,631	0,077	0,840	0,000	0,805
Media opac./pupil <6mm FU ERG[b]	rho	0,176	,354**	,257*	1,000	0,188	,236*	,218*	-0,117	-0,006	,206*	,214*
	p-value	0,099	0,001	0,015		0,073	0,024	0,038	0,267	0,954	0,049	0,040
Age	rho	0,031	,258*	,337**	0,188	1,000	-0,082	-0,136	-0,046	0,030	0,198	-0,034
	p-value	0,768	0,011	0,001	0,073		0,429	0,187	0,654	0,770	0,051	0,740
Duration of uveitis 1st ERG	rho	,286**	0,055	0,006	,236*	-0,082	1,000	,831**	-0,134	0,180	0,041	0,081
	p-value	0,005	0,595	0,954	0,024	0,429		0,000	0,194	0,080	0,696	0,433
Duration of uveitis FU ERG	rho	0,179	0,049	-0,050	,218*	-0,136	,831**	1,000	-,234*	0,002	0,134	0,029
	p-value	0,086	0,636	0,631	0,038	0,187	0,000		0,023	0,982	0,194	0,782
Syst. med 1st ERG	rho	-0,079	0,103	-0,182	-0,117	-0,046	-0,134	-,234*	1,000	,470**	-0,135	0,067
	p-value	0,445	0,319	0,077	0,267	0,654	0,194	0,023		0,000	0,186	0,510
Syst. med FU ERG	rho	0,194	0,149	0,021	-0,006	0,030	0,180	0,002	,470**	1,000	-0,014	-0,014
	p-value	0,060	0,147	0,840	0,954	0,770	0,080	0,982	0,000		0,893	0,893
Improved IT	rho	,362**	0,090	,462**	,206*	0,198	0,041	0,134	-0,135	-0,014	1,000	-0,114
	p-value	0,000	0,383	0,000	0,049	0,051	0,696	0,194	0,186	0,893		0,265
Worsened IT	rho	-0,137	,318**	-0,026	,214*	-0,034	0,081	0,029	0,067	-0,014	-0,114	1,000
	p-value	0,187	0,002	0,805	0,040	0,740	0,433	0,782	0,510	0,893	0,265	

Correlation matrix of clinical factors that had a statistically significant association with changes in the implicit time of the cone b-wave as well as systemic medication, duration of uveitis, and age. Correlations were calculated using Spearman's rho. The first ERG was measured <1 year of uveitis diagnosis and the follow-up ERG 1 year later. a) Defined as the presence of cells in the AC and/or a vitritis of ≥1+ cells and/or a FA score of >1. b) Defined as opacities of the lens/ IOL and/or vitreous opacities and/or a pupil size of <6mm. Abbreviations: ERG = electroretinogram, 1st = first, FU = follow-up, BCVA = best corrected visual acuity, AC = anterior chamber, IOL = intraocular lens, opac. = opacities, syst. = systemic, IT = implicit time of cone b-wave.

3

CHAPTER 4

PROLONGED CONE B-WAVE IMPLICIT TIME ON ELECTRORETINOGRAPHY IN RELATION TO RETINAL LAYER ANALYSIS

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ABSTRACT

Purpose

To identify possible correlations between structural changes on optical coherence tomography (OCT) and functional abnormalities on electroretinography (ERG) in uveitis patients. A characteristic ERG abnormality in uveitis is a prolonged cone b-wave implicit time (cone b-IT). We investigated the relationship between a prolonged cone b-IT and retinal layer thickness on OCT. Differences in thickness were expected to occur in the retinal layers which contain the bipolar cells: the inner plexiform layer (IPL), inner nuclear layer (INL), and outer plexiform layer (OPL).

Methods

OCT scans of 189 uveitis patients (333 eyes) were segmented with Iowa reference algorithms software. Differences in mean thickness were analyzed of ten retinal layers per region of the ETDRS grid between non-uveitis eyes and uveitis eyes with and without a prolonged cone b-IT on ERG. Spearman's rho correlation coefficients were also analyzed.

Results

The IPL was significantly thinner ($p < 0.05$, regions 2, 3, 5-9) in eyes with a prolonged cone b-IT, whereas the INL was significantly thicker ($p < 0.05$, regions 1 - 6). No differences were observed in the OPL. However, the correlation coefficients between implicit times and retinal layer thickness were weak (Spearman's rho 0.103 - 0.388, $p < 0.05$). Vitritis, FA score, and age were also weakly correlated to thickness.

Conclusions

There is a weak correlation between a prolonged cone b-IT on ERG and a thickening of the INL and thinning of the IPL. Other clinical factors including age and uveitis activity showed similar weak correlations to thickness. Therefore, differences in retinal thickness alone do not fully explain a prolonged cone b-IT.

INTRODUCTION

Uveitis is a relatively rare disease with a significant visual impact. The prevalence is estimated at 121 cases per 100.000 adults in the United States. However, uveitis causes a relatively large percentage of 10% of blindness in the working population.[1,2] Multiple treatment strategies are available with immunomodulating treatments, but still several complications can develop, including glaucoma, cystoid macula edema (CME), and retinal atrophy.[3,4]

Recently we described a characteristic abnormality on the electroretinogram (ERG) in uveitis patients: a prolonged cone b-wave implicit time (cone b-IT).[5] This ERG abnormality was present in all anatomical subtypes of uveitis and correlated to the severity of the inflammation. In most cases a prolonged cone b-IT persisted even when the inflammation had become quiescent, indicating a possible permanent loss of retinal function.[6]

We investigated a possible association between retinal function and retinal structure using automatic segmentation of the retinal layers on optical coherence tomography (OCT) images. Automatic segmentation is constantly improving, and associations between the thickness of retinal layers and clinical functional parameters have been described. For instance, in glaucoma and diabetic retinopathy a correlation was found between thinning of layer thickness and visual fields, and retinopathy stage, respectively.[7–10]

We investigated possible differences in retinal layer thickness between non-uveitis eyes and uveitis eyes with and without a prolonged cone b-IT. Because the cone b-wave is mainly generated by the ON and OFF bipolar cells,[11] we expected changes in thickness to occur in the retinal layers which correspond to the photoreceptor-bipolar cell synaps and the bipolar cells: the outer plexiform layer (OPL), inner nuclear layer (INL) and the inner plexiform layer (IPL). However, associations between the thickness of other retinal layers and the cone b-IT were investigated as well.

METHODS

Patients

We included 189 patients from a previously described cohort of 200 uveitis patients, who had an OCT on the same day as an ERG measurement and a clinical visit.[5,6] All patients were mentally competent, ≥ 18 years of age, and gave informed consent to participate. Patients were all treated at the University Medical Center Utrecht, a tertiary referral center for uveitis. Patients with uveitis associated with juvenile idiopathic

arthritis or other retinal diseases including diabetic retinopathy, retinal dystrophy, family history of retinal dystrophy, myopic degeneration, or severe media opacities were excluded.

Ethical approval was requested and obtained from the Medical Ethical Research Committee of the University Medical Center Utrecht. This study was conducted in compliance with the ethical principles of the declaration of Helsinki.

ERG measurement

ERGs were measured according to an extended protocol, with more flash strengths than the standard International Society for Clinical Electrophysiology of Vision (ISCEV) protocol.[11] For the light-adapted (LA) ERG this protocol comprised 0.3, 1.0, 3.0 and 10.0 cds/m^2 flash strengths and a 30 Hz flicker response. Dawson-Trick-Litzkow (DTL) electrodes were used as active electrodes, and an Espion E3 system with Colordome stimulator (Diagnosys LLC, Cambridge, UK) for flash stimulation. We defined the cone b-IT as prolonged when two or more consecutive flash strengths from the LA ERG were above the 95th percentile of the implicit times of a normal population.[5,11] Our reference values, and ERG results have been previously described.[5,6]

OCT retinal layer analysis

OCT images were made with Carl Zeiss Cirrus HD-OCT model 5000 (Carl Zeiss Meditec AG, Germany). C-scans were 200 x 200 x 1024 voxels and were analyzed using the Iowa Reference Algorithms (Retinal Image Analysis Lab, Iowa Institute for Biomedical Imaging, Iowa City, IA).[7,12,13] This program automatically defines the borders of ten retinal layers and calculates the mean (SD) thickness of these retinal layers per retinal region.

Figure 1 gives an overview of the ten retinal layers as seen on OCT: the retinal nerve fiber layer (RNFL), ganglion cell layer (GCL), inner plexiform layer (IPL), inner nuclear layer (INL), outer plexiform layer (OPL), outer nuclear layer (ONL), inner segment/ outer segment (IS/OS), outer segment layer (OSL), outer segment photoreceptors/ retinal pigment epithelium complex (OPR) and retinal pigment epithelium (RPE). The nine regions of which the thickness were analyzed were those of the Early Treatment Diabetic Retinopathy Study (ETRDRS) grid.

Statistical analysis

R Studio (R studio, 2019, version 3.6.0) was used for statistical analyses. The distribution of the automatically segmented thickness analysis was evaluated with Shapiro-Wilk tests, Q-Q plots, histograms and boxplots. Extreme outliers (Tukey's k = 3) were
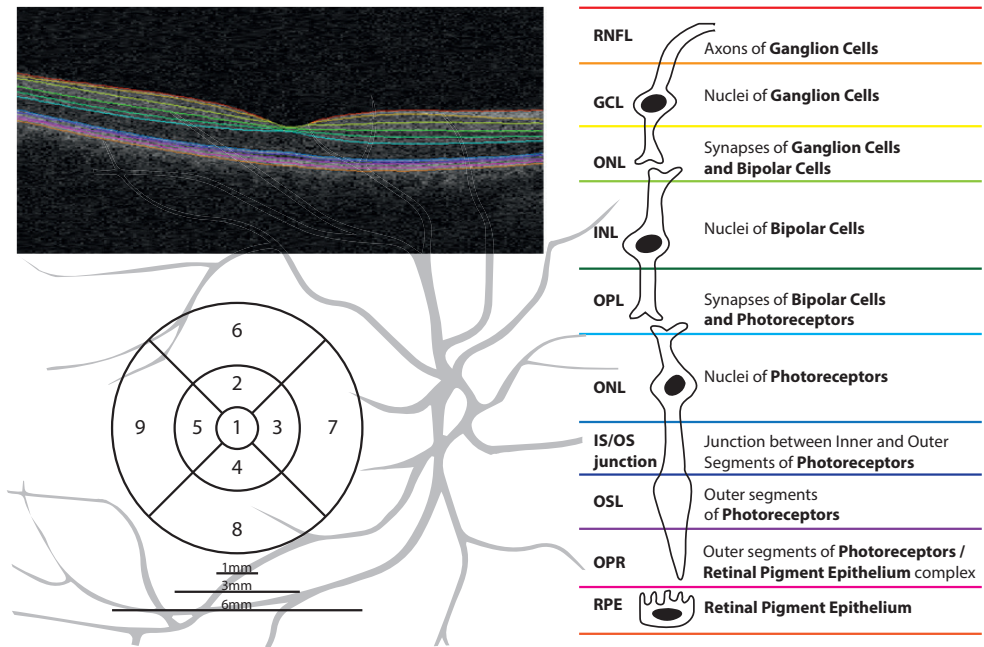
Figure 1: Example of the ten segmented retinal layers and the Early Treatment Diabetic Retinopathy Study (ETDRS) grid. On the right a schematic representation of the different cell types per layer. Abbreviations: RNFL = retinal nerve fiber layer, GCL = ganglion cell layer, IPL = inner plexiform layer, INL = inner nuclear layer, OPL = outer plexiform layer, ONL = outer nuclear layer, IS/OS junction = inner segment/outer segment junction, OSL = outer segment layer, OPR = outer segment photoreceptors/retinal pigment epithelium complex (OPR) and retinal pigment epithelium (RPE).

identified, and the segmentation of these OCT images was manually checked and corrected when necessary.

After manual correction, the thickness analysis was performed again and the thicknesses of the different retinal layers were compared between uveitis eyes with a prolonged cone b-IT, uveitis eyes with a normal cone b-IT, and eyes with no uveitis (of patients with a unilateral uveitis). Also, correlations between the implicit times of the cone b-wave of the different flash strengths (0.3, 1.0, 3.0 and 10.0 cds/m^2) in ms and thicknesses in microns were investigated. We considered p-values of < 0.05 as statistically significant. All tests were 2-tailed.

Next, layer thickness were investigated in relation to clinical parameters that were associated with a prolonged cone b-IT including: fluorescein angiography (FA) scores (graded according to the Angiography Scoring for Uveitis Working Group (ASUWOG)),[14] the presence of cells in the anterior chamber (trace or more), or vitreous (1+ or more), as graded according the standardization of uveitis nomenclature (SUN) criteria,[15] and age, using Spearman's rho coefficient.

RESULTS

Patient population

A total of 372 eyes of 189 patients were included. Two of the initial 200 uveitis patients of our cohort were excluded because the OCT scan was not performed on the same day as the ERG. Seven patients were excluded because their OCT scan was performed with a different OCT device (Heidelberg). Lastly, two patients were excluded because the algorithm could not properly segment the retinal layers in their eyes, due to a severe macula pucker or CME.

In five patients with a bilateral uveitis, one eye was excluded because the algorithm could not properly segment the retinal layers of one eye. There were 40 uveitis patients with a unilateral uveitis. In one of these, there was only an OCT scan available of the uveitis eye. This enabled us to investigate 39 non-uveitis eyes of patients with a unilateral uveitis.

Table 1 gives an overview of the patient characteristics and diagnoses. Of the 333 uveitis eyes, 142 eyes (42.6%) had a prolonged cone b-IT. None of the non-uveitis eyes (n = 39) of patients with a unilateral uveitis had a prolonged cone b-IT. Patients with a prolonged cone b-IT were on average older (median 61.2 (IQR 51.2 – 70.1) years) compared to patients with a normal cone b-IT (median 44.8 (IQR 33.7 – 55.7) years) and patients with unilateral uveitis (median 47.1 (IQR 32.4 -56.3) years)) (p < 0.001). There was no differences based on gender (p = 0.320). Most patients had a bilateral uveitis (n = 149 (78.8 %)). A prolonged cone b-IT was equally observed in unilateral and bilateral uveitis. There were no differences between eyes with and without a prolonged cone b-IT and uveitis diagnosis (p = 0.198), or anatomical localization of uveitis (p = 0.802).

Abbreviations: N = number, IQR = interquartile range, MS = multiple sclerosis, HLA B27 = human leucocyte antigen B27, VKH = Vogt-Koyanagi-Harada.

Table 1: Patient characteristics

	Patients (n=189)
Male gender, N (%)	57 (30.2)
Age, median [IQR]	53.65 [39.16, 64.07]
Bilateral uveitis, N (%)	149 (78.8)
Diagnosis, N (%)	
Unknown cause	76 (40.2)
Sarcoidosis	29 (15.3)
Chorioretinitis	27 (14.3)
Birdshot	26 (13.8)
HLA-B27	14 (7.4)
MS	7 (3.7)
Behçet	5 (2.6)
VKH	5 (2.6)

OCT thickness analysis

Table 2 shows in shaded grey the results of the IPL, INL, and OPL, the layers which encompass the bipolar cells. In eyes with a prolonged cone b-IT, the mean thickness of the IPL was significantly thinner in seven regions of the ETDRS grid (regions 2, 3, 5 - 9). In contrast, the INL layer was significantly thicker in the six central regions of the ETDRS grid (regions 1 - 6) in eyes with a prolonged cone b-IT. No differences were observed in the OPL.

Table 3 gives an overview of Spearman's rho correlation coefficients of implicit times in ms of the different flash strengths in relation to the thickness in microns of the IPL and INL. In line with the findings in Table 2, these correlations between thickness and implicit time were often statistically significant, but the correlation coefficients were weak ranging between 0.103 and 0.388. Weak correlations were also found between the thickness of the INL and IPL and clinical parameters that were previously found to be associated with a prolonged cone b-IT (Table 4).[5]

Besides the retinal layers which contain the bipolar cells, other layers also showed significant differences in thickness in eyes with a prolonged cone b-IT (Table 2). The retinal nerve fiber layer (RNFL) was significantly thicker (Table 2). Glaucoma, a common complication of uveitis, was investigated as a possible confounder for the differences in the RNFL thickness. Eyes with glaucoma (n = 67 eyes) had a significantly thicker RNFL in regions 1, 7 and 9 and a thinner GCL and IPL in regions 2 to 9.

Eyes with glaucoma also had a thicker INL (region 3, 7), similarly to eyes with a prolonged cone b-IT. However, when eyes with glaucoma were excluded and a comparison was made between eyes with a prolonged cone b-IT, a normal cone b-IT, and non-uveitis eyes, the IPL was still significantly thinner and the INL was thicker in eyes with a prolonged cone b-IT. The other layers also showed similar outcomes when glaucoma eyes were excluded.

The outer retinal layers showed significant differences as well (Table 2). The largest differences were observed between the non-uveitis eyes and uveitis eyes with a prolonged cone b-IT. The results of the uveitis eyes with a normal cone b-IT lay in between, but their retinal layer thicknesses were closer to those of the non-uveitis eyes. When the non-uveitis eyes were excluded, and only the differences between uveitis eyes with and without a prolonged cone b-IT were compared, similar outcomes were observed. Regarding the layers containing bipolar cells, the only different outcome was that the INL was no longer significantly thicker in region 3.

Table 2: Thickness analysis of the retinal layers of the different regions of the ETDRS grid of uveitis eyes with and without a prolonged cone b-wave, and non-uveitis eyes.

		Mean thickness	region 1	region 2	region 3	region 4
Ganglion cells	**RNFL**	no uveitis	5.20 [3.42, 8.28]	22.98 [21.38, 26.17]	27.29 [24.49, 30.19]	25.38 [21.91, 28.73]
		uveitis normal b-IT	6.69 [3.86, 13.09]	24.74 [21.55, 30.91]	29.09 [26.03, 33.30]	24.84 [21.11, 29.89]
		prolonged b-IT	11.48 [7.50, 20.13]	28.45 [23.67, 36.21]	34.58 [28.65, 41.81]	28.59 [23.13, 35.55]
		p-value	**<0.001**	**<0.001**	**<0.001**	**<0.001**
	GCL	no uveitis	17.16 [13.60, 22.48]	52.35 [45.59, 56.27]	54.31 [48.99, 58.54]	52.75 [47.95, 56.11]
		uveitis normal b-IT	18.29 [13.48, 23.40]	50.48 [45.12, 55.94]	52.50 [47.16, 58.31]	51.20 [44.81, 57.44]
		prolonged b-IT	18.80 [12.40, 29.87]	49.66 [38.89, 57.19]	51.75 [39.96, 57.03]	49.78 [40.78, 58.82]
		p-value	0.517	0.233	0.098	0.511
Bipolar cells	**IPL**	no uveitis	26.92 [24.08, 28.94]	38.53 [35.66, 40.46]	41.64 [36.03, 44.48]	37.24 [35.11, 40.65]
		uveitis normal b-IT	26.59 [22.47, 29.50]	36.39 [33.23, 40.78]	39.25 [35.13, 43.77]	36.67 [32.34, 40.42]
		prolonged b-IT	27.11 [23.17, 30.27]	35.02 [30.47, 40.00]	36.70 [31.84, 41.56]	35.86 [30.49, 39.75]
		p-value	0.443	**0.004**	**0.006**	0.175
	INL	no uveitis	20.83 [17.05, 23.17]	36.47 [33.91, 39.89]	40.60 [37.70, 43.02]	37.62 [35.45, 41.20]
		uveitis normal b-IT	21.73 [17.67, 27.61]	38.04 [34.41, 41.94]	42.61 [39.02, 45.82]	38.75 [34.84, 43.00]
		prolonged b-IT	26.20 [21.17, 37.83]	40.75 [36.11, 47.26]	43.42 [38.41, 48.54]	41.35 [36.74, 48.85]
		p-value	**<0.001**	**<0.001**	**0.043**	**<0.001**
	OPL	no uveitis	23.92 [20.34, 26.66]	27.05 [24.71, 30.66]	29.04 [26.52, 34.81]	30.44 [26.00, 35.96]
		uveitis normal b-IT	22.20 [17.97, 26.68]	27.96 [24.91, 32.59]	29.20 [25.57, 33.49]	27.93 [25.04, 34.69]
		prolonged b-IT	22.93 [17.18, 28.62]	28.49 [24.82, 34.42]	29.25 [24.24, 34.73]	27.78 [24.97, 34.32]
		p-value	0.466	0.647	0.509	0.286
Photoreceptors	**ONL**	no uveitis	115.53 [108.11, 124.16]	99.20 [89.18, 105.66]	92.44 [83.58, 97.56]	97.53 [86.02, 102.44]
		uveitis normal b-IT	117.11 [105.54, 126.93]	97.31 [84.84, 107.96]	91.62 [77.42, 98.02]	93.94 [80.00, 104.91]
		prolonged b-IT	114.15 [100.79, 127.75]	92.88 [78.62, 104.73]	86.14 [75.37, 96.14]	94.59 [81.83, 104.61]
		p-value	0.652	0.150	0.105	0.865
	IS/ OS	no uveitis	11.47 [11.13, 11.85]	10.45 [9.96, 10.71]	10.20 [9.82, 10.46]	10.35 [10.06, 10.62]
		uveitis normal b-IT	11.29 [10.86, 11.81]	10.42 [10.07, 10.88]	10.16 [9.81, 10.69]	10.40 [10.00, 10.79]
		prolonged b-IT	11.18 [10.64, 12.29]	10.68 [10.03, 12.03]	10.39 [9.73, 11.58]	10.59 [10.02, 11.62]
		p-value	0.167	0.058	0.137	**0.049**
	OSL	no uveitis	20.40 [19.59, 21.72]	14.22 [12.07, 17.73]	12.93 [11.47, 16.62]	13.94 [12.53, 16.75]
		uveitis normal b-IT	20.75 [18.96, 22.44]	14.99 [13.00, 18.78]	14.66 [12.39, 19.19]	15.10 [12.91, 18.55]
		prolonged b-IT	21.03 [19.28, 23.80]	17.03 [14.35, 20.50]	16.85 [13.28, 20.63]	16.88 [13.56, 20.07]
		p-value	0.337	**<0.001**	**0.001**	**0.013**
	OPR	no uveitis	20.53 [17.85, 22.60]	21.69 [16.00, 24.06]	22.60 [16.84, 24.40]	21.78 [17.92, 24.27]
		uveitis normal b-IT	19.59 [16.52, 21.84]	20.41 [15.66, 23.30]	20.51 [14.96, 23.65]	19.94 [15.86, 22.67]
		prolonged b-IT	15.73 [12.05, 19.20]	14.09 [11.06, 18.73]	14.34 [11.96, 19.03]	15.18 [11.88, 19.22]
		p-value	**<0.001**	**<0.001**	**<0.001**	**<0.001**
RPE	**RPE**	no uveitis	14.87 [14.73, 15.11]	14.96 [14.68, 15.09]	14.88 [14.66, 15.07]	14.93 [14.73, 15.12]
		uveitis normal b-IT	14.85 [14.52, 15.09]	14.84 [14.59, 15.07]	14.80 [14.59, 15.01]	14.74 [14.48, 15.05]
		prolonged b-IT	14.79 [14.42, 15.03]	14.86 [14.52, 15.06]	14.82 [14.60, 15.00]	14.75 [14.53, 15.00]
		p-value	0.222	0.456	0.540	0.077

Results of the mean thickness analysis of the RNFL, GCL, IPL, INL, OPL, ONL, IS/OS junction, OSL, OPR and RPE of eyes with no uveitis (n = 39; these were of patients with a unilateral uveitis and all had a normal cone b-IT), uveitis eyes with a prolonged cone b-IT (n = 142 eyes), and uveitis eyes with a normal cone b-IT (n = 191 eyes). Results are presented per region of the Early Treatment Diabetic Retinopathy Study (ETDRS) grid using the Iowa Reference Algorithms. Data are presented as median [IQR]. Significances were tested with Kruskal-Wallis test. All tests were two-tailed. Abbreviations: b-IT

region 5	region 6	region 7	region 8	region 9
27.47 [25.62, 30.64]	19.20 [16.64, 49.34]	39.19 [36.36, 45.15]	41.93 [18.43, 53.19]	42.84 [38.87, 46.77]
29.74 [25.44, 33.67]	30.71 [19.15, 55.08]	43.83 [38.31, 50.71]	29.58 [19.05, 56.39]	46.95 [39.94, 53.84]
33.56 [28.05, 40.93]	39.06 [23.39, 61.77]	50.36 [41.65, 62.17]	33.48 [23.04, 63.87]	53.09 [43.27, 60.59]
<0.001	**0.001**	**<0.001**	**0.040**	**<0.001**
55.21 [47.86, 58.28]	27.86 [26.40, 31.45]	26.34 [23.44, 28.23]	30.51 [27.14, 32.70]	24.58 [21.79, 26.78]
52.95 [45.70, 58.23]	30.28 [26.12, 34.26]	25.17 [22.75, 30.21]	29.25 [25.54, 34.32]	23.92 [20.66, 27.23]
49.26 [36.74, 57.43]	29.13 [23.99, 34.52]	25.09 [20.32, 28.43]	29.98 [25.05, 36.06]	22.81 [19.87, 25.97]
0.026	0.336	0.168	0.910	0.220
40.83 [36.88, 43.92]	38.48 [36.56, 42.23]	34.96 [32.72, 36.75]	36.97 [33.70, 42.05]	33.11 [31.26, 37.04]
39.19 [34.23, 43.31]	37.82 [33.97, 41.39]	33.89 [30.42, 37.37]	36.82 [32.47, 41.27]	33.74 [30.29, 37.29]
36.59 [32.80, 41.26]	34.43 [31.59, 37.86]	32.26 [28.76, 35.35]	34.62 [30.01, 38.08]	31.43 [28.34, 34.77]
0.003	**<0.001**	**0.001**	**0.002**	**0.001**
40.83 [37.40, 43.62]	30.04 [28.52, 32.11]	29.06 [26.75, 31.13]	31.35 [29.82, 32.79]	27.52 [25.97, 29.87]
41.68 [38.41, 44.97]	32.23 [29.45, 35.14]	30.09 [27.77, 33.05]	31.92 [29.29, 35.30]	29.26 [27.10, 32.16]
43.45 [38.63, 48.94]	32.09 [28.48, 36.15]	30.00 [26.82, 33.74]	32.16 [28.64, 37.64]	29.66 [25.99, 33.31]
0.014	**0.042**	0.117	0.364	0.068
27.93 [26.33, 30.90]	25.66 [24.02, 27.29]	24.78 [23.39, 26.82]	26.51 [24.70, 28.87]	24.26 [23.01, 25.51]
27.67 [25.12, 31.63]	26.22 [24.09, 28.45]	24.88 [23.15, 26.70]	26.10 [23.92, 29.02]	24.67 [22.93, 26.64]
27.42 [24.77, 34.04]	24.81 [23.20, 28.54]	24.55 [22.55, 27.21]	25.82 [23.72, 28.53]	24.50 [22.60, 27.79]
0.925	0.071	0.486	0.330	0.636
92.46 [83.78, 98.11]	79.12 [69.81, 83.61]	82.14 [76.35, 85.62]	79.66 [70.51, 84.33]	73.86 [68.66, 77.67]
90.15 [77.87, 98.95]	77.06 [69.44, 84.68]	79.84 [73.28, 86.61]	76.05 [67.83, 83.81]	71.71 [65.09, 78.00]
86.60 [73.77, 95.27]	75.68 [67.08, 83.72]	77.58 [71.40, 84.24]	75.70 [69.43, 82.61]	70.33 [64.48, 77.02]
0.044	0.382	0.064	0.357	0.221
10.08 [9.84, 10.48]	10.10 [9.68, 10.60]	9.85 [9.66, 10.24]	9.87 [9.53, 10.16]	10.12 [9.64, 11.32]
10.24 [9.88, 10.71]	10.31 [9.86, 11.41]	10.00 [9.60, 10.67]	10.31 [9.79, 11.08]	10.72 [9.91, 12.83]
10.44 [9.82, 12.03]	11.15 [10.06, 13.80]	10.47 [9.95, 12.30]	10.93 [10.16, 13.31]	11.73 [10.15, 14.23]
0.042	**<0.001**	**<0.001**	**<0.001**	**<0.001**
13.45 [11.76, 16.45]	13.99 [10.88, 17.18]	15.81 [10.66, 18.86]	13.43 [10.64, 18.16]	16.83 [12.41, 19.13]
14.49 [12.34, 18.03]	15.49 [12.24, 19.16]	16.12 [12.08, 20.28]	15.52 [12.67, 19.16]	16.78 [13.73, 19.40]
15.96 [13.39, 19.50]	16.16 [13.39, 19.04]	16.90 [13.76, 20.43]	15.84 [13.51, 18.97]	15.70 [13.48, 18.25]
0.007	0.065	0.136	0.080	0.258
21.61 [17.15, 23.64]	18.70 [12.91, 23.08]	18.98 [13.62, 23.40]	19.50 [15.02, 22.81]	15.25 [12.64, 17.71]
18.71 [14.12, 22.45]	16.07 [12.68, 20.27]	14.86 [12.42, 20.81]	15.76 [12.38, 20.63]	13.36 [11.30, 16.70]
13.59 [10.58, 17.83]	12.70 [10.98, 16.07]	13.06 [11.10, 17.07]	13.05 [11.10, 15.78]	11.75 [10.11, 15.21]
<0.001	**<0.001**	**<0.001**	**<0.001**	**<0.001**
14.83 [14.71, 15.06]	14.92 [14.55, 15.18]	14.84 [14.68, 15.16]	14.85 [14.56, 15.05]	14.79 [14.54, 15.02]
14.81 [14.54, 15.04]	14.80 [14.52, 15.00]	14.84 [14.60, 15.06]	14.72 [14.50, 14.97]	14.75 [14.52, 14.98]
14.79 [14.45, 15.04]	14.77 [14.57, 15.00]	14.86 [14.64, 15.11]	14.75 [14.52, 15.05]	14.79 [14.57, 15.04]
0.439	0.669	0.634	0.543	0.600

= implicit time of the cone b-wave on electroretinogram, RNFL = Retinal Nerve Fiber Layer, GCL = ganglion cell layer, IPL = inner plexiform layer, INL = inner nuclear layer, OPL = outer plexiform layer, ONL = outer nuclear layer, IS/OS junction = inner segment outer segment junction, OSL = outer segment layer, OPR = outer segment photoreceptors/retinal pigment epithelium complex, RPE = retinal pigment epithelium, IQR = inter quartile range.

Table 3: Correlations between different flash strengths of the implicit time of the cone b-wave and mean thickness in microns of the inner plexiform layer and inner nuclear layer of the different regions of the ETDRS grid.

	Cone b-IT		region 1	region 2	region 3	region 4	region 5	region 6	region 7	region 8	region 9
Inner plexiform layer	0.3 cds/m²	rho	,109*	-,185**	-,209**	-0,101	-,203**	-,338**	-,262**	-,261**	-,272**
		p-value	0,036	0,000	0,000	0,053	0,000	0,000	0,000	0,000	0,000
	1.0 cds/m²	rho	0,085	-,184**	-,190**	-0,086	-,182**	-,343**	-,261**	-,239**	-,265**
		p-value	0,103	0,000	0,000	0,098	0,000	0,000	0,000	0,000	0,000
	3.0 cds/m²	rho	0,043	-,265**	-,258**	-,159**	-,246**	-,388**	-,296**	-,296**	-,296**
		p-value	0,409	0,000	0,000	0,002	0,000	0,000	0,000	0,000	0,000
	10.0 cds/m²	rho	,115*	-,158**	-,214**	-,125*	-,166**	-,322**	-,272**	-,276**	-,267**
		p-value	0,027	0,002	0,000	0,016	0,001	0,000	0,000	0,000	0,000
Inner nuclear layer	0.3 cds/m²	rho	,313**	,257**	,150**	,196**	,141**	0,064	0,017	0,040	0,039
		p-value	0,000	0,000	0,004	0,000	0,006	0,219	0,751	0,447	0,457
	1.0 cds/m²	rho	,332**	,215**	,113*	,204**	,122*	0,028	0,002	0,030	0,025
		p-value	0,000	0,000	0,030	0,000	0,019	0,589	0,964	0,563	0,626
	3.0 cds/m²	rho	,274**	,204**	0,087	,148**	0,0764	0,006	-0,020	0,004	0,003
		p-value	0,000	0,000	0,095	0,004	0,141	0,905	0,697	0,943	0,954
	10.0 cds/m²	rho	,301**	,228**	,131*	,175**	,130*	0,045	0,014	0,025	0,028
		p-value	0,000	0,000	0,011	0,001	0,012	0,386	0,794	0,631	0,586

Abbreviations: cone b-IT = implicit time of the cone b-wave, ETDRS = Early Treatment Diabetic Retinopathy Study.

Table 4: Correlations between clinical parameters and mean thickness in microns of the inner plexiform layer and inner nuclear layer of the different regions of the ETDRS grid.

	Clinical parameters		region 1	region 2	region 3	region 4	region 5	region 6	region 7	region 8	region 9
Inner plexiform layer	Cells AC	rho	-0,038	0,003	0,002	-0,005	-0,030	-0,010	0,013	-0,012	-0,009
		p-value	0,469	0,950	0,972	0,929	0,561	0,845	0,806	0,818	0,868
	Vitritis	rho	,124*	0,100	0,073	,106*	0,086	0,019	0,092	0,086	0,070
		p-value	0,018	0,058	0,165	0,044	0,100	0,722	0,079	0,101	0,183
	FA score	rho	0,010	-,187**	-,141*	-0,094	-,178**	-,194**	-,119*	-0,090	-0,087
		p-value	0,857	0,001	0,012	0,098	0,002	0,001	0,035	0,111	0,123
	Age	rho	0,093	-,228**	-,311**	-,196**	-,283**	-,380**	-,390**	-,375**	-,371**
		p-value	0,073	0,000	0,000	0,000	0,000	0,000	0,000	0,000	0,000
Inner nuclear layer	Cells AC	rho	,116*	,129*	,120*	,127*	,123*	,108*	,115*	0,065	,106*
		p-value	0,027	0,013	0,022	0,015	0,019	0,040	0,029	0,218	0,042
	Vitritis	rho	,164**	,193**	,161**	,124*	,174**	,116*	0,097	,108*	,117*
		p-value	0,002	0,000	0,002	0,018	0,001	0,027	0,065	0,039	0,026
	FA score	rho	,207**	,222**	,166**	,151**	,151**	,148**	,120*	,148**	,156**
		p-value	0,000	0,000	0,003	0,007	0,008	0,009	0,034	0,009	0,006
	Age	rho	,268**	,226**	,108*	,175**	,122*	-0,061	-,173**	-,110*	-,127*
		p-value	0,000	0,000	0,037	0,001	0,019	0,244	0,001	0,034	0,014

Abbreviations: cone b-IT = implicit time of the cone b-wave, ETDRS = Early Treatment Diabetic Retinopathy Study, AC = anterior chamber, FA = fluorescein angiography.

DISCUSSION

This study illustrates an association between an abnormal retinal function as measured by ERG, and a difference in retinal thickness on OCT. We observed that eyes with a prolonged cone b-IT, had differences in thickness in the retinal layers which correspond to the bipolar cells, i.e. a thinner IPL and a thicker INL.

However, the correlation between the implicit times and thickness was weak and other factors, such as age and uveitis activity, were also correlated to the observed differences in thickness. Therefore, the prolonged cone b-IT is probably multifactorial and a change in thickness can only partly explain the observed differences. This study is to our knowledge the first that investigated the thickness of specific retinal layers in uveitis in relation to retinal function as measured by the ERG.

The thickening of the INL in uveitis could be due to subtle intraretinal edema or inflammatory deposits. One can speculate that this impairs phototransmission and therefore results in a prolonged cone b-IT on the ERG. The thinner IPL may indicate a loss of cells, which may lead to permanent damage and possibly even retinal atrophy.

Changes in retinal thickness in eyes with a prolonged cone b-IT were also observed in other layers than those which contain the bipolar cells. Except for the OPR, most layers were thickened. This is contrary to many retinal dystrophies where a loss of function is associated with a thinning of retinal layers, indicating a loss of cells.[16]

In line with previous reports we observed a weak and mostly negative correlation between age and retinal layer thickness.[17,18] Interestingly, in the INL the correlation coefficient was positive for the inner regions, but negative for the outer regions. This indicates that for the inner regions of the INL, age may be a confounding factor in the current study. Age also affects implicit time, but differences in age cannot fully explain the observed prolonged cone b-IT because uveitis affects the implicit time more than age alone.[5]

Future research is necessary to investigate if anatomical or functional changes can be first observed in uveitis. To answer this question, OCT and ERG measurements have to be repeatedly done from the very first start of uveitis symptoms. Preferably, cone ERGs should be recorded with an extended protocol, because the prolonged cone b-IT is often best detected at lower stimulus strengths than at the standard light adapted 3.0 stimulus flash.[5]

Several studies compared the thickness of retinal layers during the active phase of uveitis to the thickness in the inactive phase, but these did not investigate the ERG. Because a prolonged cone b-IT is associated with a more active uveitis,[5,6,19] some comparisons with literature can be made.

Yamamoto et al. described that in Vogt-Koyanagi-Harada uveitis the RNFL and the inner retina (RNFL, GCL and IPL combined) were significantly thicker in the active stage of the disease.[20] The correlation between an active uveitis and a thicker RNFL has also been described in several other studies, and even in uveitis eyes with glaucoma.[21–24] In the current study, eyes with a prolonged cone b-IT had also a thicker RNFL compared to eyes with a normal cone b-IT. In our study vitritis and FA score were also correlated to the RNFL (data not shown).

The combination of a thinner IPL and a thicker INL was not found in other studies, because these layers were not analyzed separately. When we analyzed the combined thickness of the IPL and INL, a significant difference was only observed in region 1 (thicker) and region 6 (thinner). In anterior uveitis Lee et al. also found no difference in the combined thickness of the IPL and GCL between the active and inactive phase of uveitis.[24] This highlights that it is important to evaluate the retinal layers individually, because otherwise subtle changes can be overlooked.

We saw a clearer association between a prolonged cone b-IT and a thinner OPR. In birdshot uveitis, similar findings were described, i.e. birdshot eyes had a thinner photoreceptor outer segment, which was even more affected in eyes with an abnormal ERG, which may be an indication of a more severe form of birdshot uveitis.[25]

A limitation of this study was that no comparison was made in retinal layer thickness between uveitis patients and healthy controls, because we do not have a database with normal values for the thickness of every retinal layer. Non-uveitis eyes of patients with unilateral uveitis are less suitable for comparison, because it may be difficult to be absolutely certain that subtle subclinical changes are absent. However, analysis of these non-uveitis eyes showed that the biggest differences in retinal thickness were between non-uveitis eyes and uveitis eyes with a prolonged cone b-IT.

Another limitation of this study was that OCT scans were analyzed using cube data. These have a lower resolution than high definition scans. However, the software program which we used only facilitates the export of thickness analysis data of scans which correspond with a 3D image. As we used a large sample size, we do not expect that a change in thickness would be in favor of one layer or the other, and therefore we expect that these results are representative.

Besides the quality of the scans, it is also important to note that the ERG measures the response of the entire retina, whereas the OCT only evaluates the central retina of approximately 6 x 6 mm. This should be kept in mind when imaging results are investigated in relation to functional data. Therefore, the thickening of the INL might be of less consequence on the ERG, because it was only observed in the central regions of the ETDRS grid and the macula contributes only minimally to the full-field ERG.[26] Contrary to the INL, the thinning of the IPL was present in the peripheral

regions on the ETDRS grid. If this thinning continues towards periphery, it may be of much greater influence on the cone b-IT.

In summary, we have shown a correlation between structural and functional changes in uveitis. The observed changes in the INL and IPL can help in our understanding of the possible causes of a prolonged cone b-IT on ERG. However, the observed correlation was weak, and other clinical factors including age and uveitis activity showed similar weak correlations to the thickness of retinal layers. Thus, there is a relation between function and structure, but a prolonged cone b-IT cannot be fully explained by differences in retinal thickness on OCT alone. Because the ERG investigates the retina in a different manner than the OCT and the observed correlations were weak, there may be an additional role for the ERG in the understanding of the effects of uveitis on the retina. Further research is necessary to evaluate if changes occur first on the ERG or on OCT, in order to assess if the ERG can be used for prognosis and treatment decisions in uveitis.

ACKNOWLEDGEMENTS

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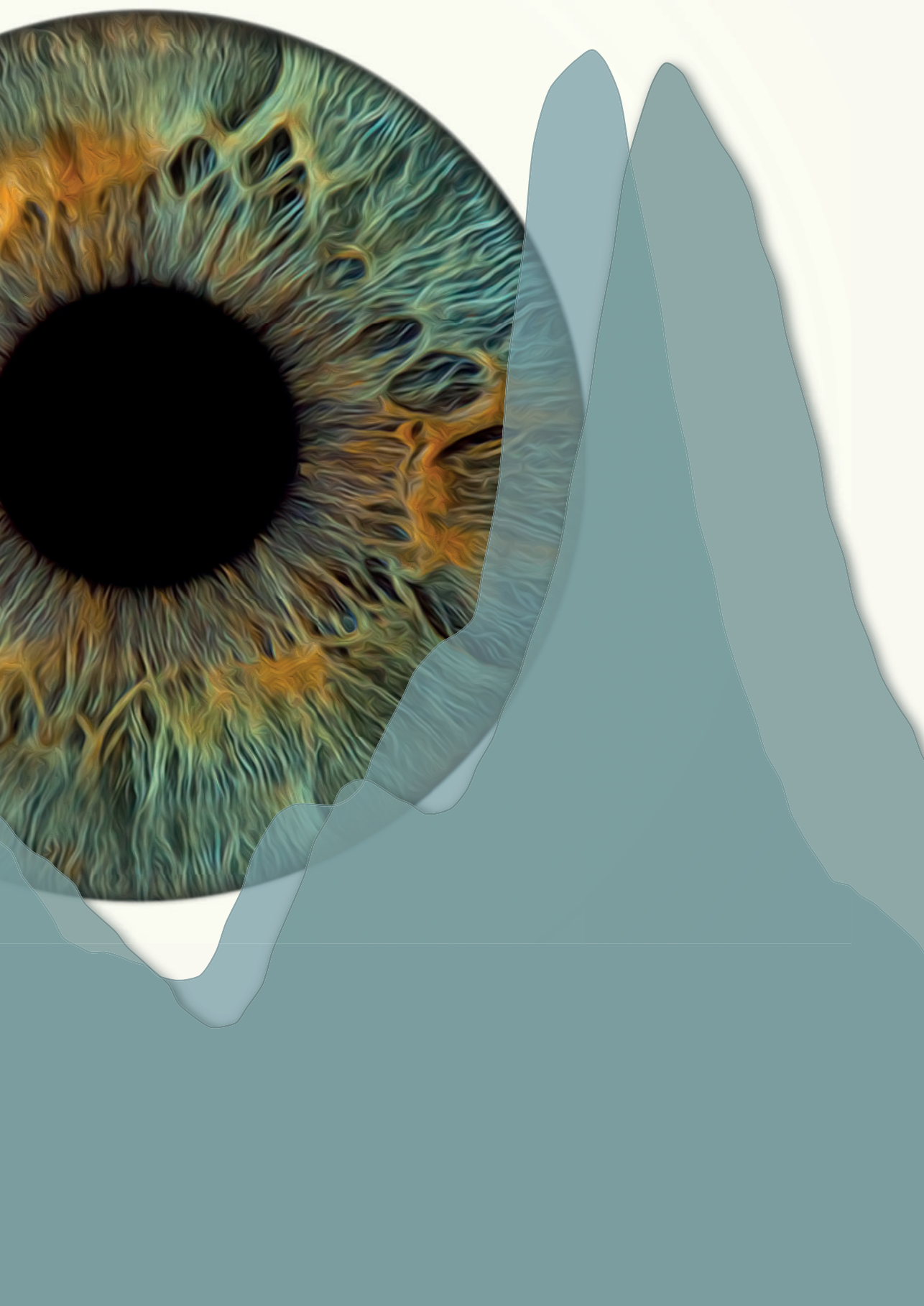
CHAPTER 5

ELECTRORETINOGRAM ABNORMALITIES IN NON-ANTERIOR CHILDHOOD UVEITIS

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ABSTRACT

Purpose

A major point of concern in uveitis is the development of irreversible retinal changes after inflammation. In this study, we assess how non-anterior childhood uveitis affects retinal function using full field electroretinography (ERG).

Methods

Cross-sectional study. ERGs of 63 uveitis eyes (33 children) were measured according to extended International Society for Clinical Electrophysiology of Vision (ISCEV) protocols. ERG abnormalities were investigated in relation to the following clinical parameters: demographics, uveitis characteristics, including severity of inflammation, treatment, best corrected visual acuity (BCVA), cystoid macular edema (CME) on optical coherence tomography (OCT) and fluorescein angiography score.

Results

The ERG showed abnormalities in 34 eyes (54%). The most frequent ERG abnormalities were prolonged implicit times of the cone b-wave (37%; n = 23/63) and an abnormal 30 Hz flicker response (implicit time and/or amplitude) (33%; n = 21/63). Factors associated with these ERG abnormalities were CME ($p = .021$) and 3+ vitreous cells ($p = .021$). BCVA in eyes with and without these ERG abnormalities did not statistically differ, and was relatively good (median 0.05 LogMAR, IQR 0.00 - 0.15 LogMAR).

Conclusion

The ERG is frequently affected in childhood uveitis indicating a global retinal dysfunction. ERG abnormalities seem to be associated with a more severe posterior segment inflammation and a younger age. If an association between ERG abnormalities and long term visual outcome can be made in the future, these early ERG findings during the course of childhood uveitis have significance for treatment strategies.

INTRODUCTION

Non-anterior, non-infectious uveitis is a serious disease with unilateral blindness developing in up to 19% of children, despite intensive immunomodulating treatment. This blindness is often caused by complications such as cystoid macular edema (CME) and glaucoma.[1–3]

In addition, after a prolonged course of inflammation, patients with uveitis may develop thinning of the retina with "retinal dystrophy-like" changes and dragged disk vessels.[3–5]

To gain a better understanding of the effects of uveitis on retinal function, a full field (Ganzfield) electroretinogram (ERG) can be used. The ERG objectively measures retinal function and may therefore provide useful additional information to imaging techniques.

Electroretinography abnormalities have been described in various uveitis entities. Particularly in birdshot chorioretinopathy (BSCR),[5,6] the ERG is used for monitoring disease activity and treatment, but it may also be useful in other uveitis entities.[5,7,8] However, there are few studies on ERG abnormalities in childhood uveitis and knowledge is still lacking on the effects of uveitis on retinal function in children.[9]

In this study, we retrospectively analyzed ERG abnormalities in children with a non-infectious and non-anterior uveitis. We correlate their ERG abnormalities to clinical parameters and investigate the value of the ERG as an additional tool to objectively assess retinal damage in childhood uveitis.

METHODS

Study population

We included 33 patients (63 eyes) with a non-infectious, non-anterior uveitis. The median age at diagnosis was 8.9 years (range 3.5 - 14.6 years). All patients were seen at the ophthalmology department of the University Medical Centre Utrecht (UMC Utrecht). Here we perform an ERG as part of the clinical work-up if no obvious underlying cause for uveitis is found, to exclude a retinal dystrophy.[10] In case of doubt regarding the ERG abnormalities, such as reduction of amplitudes, DNA was tested for retinal dystrophy mutations, which was negative (N = 3). Furthermore, none of the patients had alterations suggestive of a retinal dystrophy on their latest optical coherence tomography (OCT) and/or visual fields. All patients had an ERG examination between May 2015 and December 2016.

The uveitis diagnosis was based on the Standardization of Uveitis Nomenclature (SUN) criteria[11] and made by an ophthalmologist specialized in uveitis. Electroretinograms and their medical charts were retrospectively reviewed.

This study was conducted in compliance with the ethical principles of the declaration of Helsinki. Ethical approval was requested and obtained from the Medical Ethical Research Committee of the UMC Utrecht. Depending on the age of the patients, we obtained consent from the patients themselves, or their parents, or both.

ERG analysis

ERGs were measured incorporating the International Society for Clinical Electrophysiology of Vision (ISCEV) standards.[12] Dawson-Trick-Litzkow (DTL) electrodes were used as corneal electrodes. An Espion E3 system with ColorDome Stimulator (Diagnosys LLC, Cambridge, UK) was used for flash stimulation.

We measured an extended ISCEV series, consisting of stimulus strengths that increase with approximately 0.5 log units and range from 0.0001 - 30.0 cds/m^2 for the dark-adapted ERG (DA; rod and combined rod/cone) and from 0.3 - 10.0 cds/m^2 for the light-adapted ERG (LA; cone), including a 30 Hz flicker response (LA; cone).

The ERGs of patients were compared to healthy controls with the same age range as uveitis patients (5 - 22 years) (N = 50). These ERGs were measured with the same equipment and protocols and provided by the Rotterdam Eye Hospital and Bartiméus (Bartiméus Diagnostic Centre for complex visual disorders, Zeist). An ERG was considered abnormal if amplitudes were below the 2.5th percentile, or implicit times were above the 97.5th percentile, or both.

We categorized the ERG abnormalities based on the measuring conditions ((LA 0.3 - 10.0 cds/m^2 (cone response), 30 Hz flicker response (cone response), DA 0.0001 - 0.01 cds/m^2 (rod response) or DA 3.0 - 30.0 cds/m^2 (combined rod cone response)) and which part of the response was abnormal (a-wave, b-wave, amplitudes or implicit times). We did not classify DA 0.03 - 1.0 cds/m^2, as stimulus strengths lay between rod and combined rod-cone responses.

All ERGs could be used for analyses. It is our experience that in a child-friendly environment, it is possible to measure an ERG properly, even in small children.

Clinical parameters

Medical records were reviewed for age, gender, medical history and age at onset of uveitis (defined as age at the date of diagnosis by a (referring) ophthalmologist). Using the outpatient visit closest to ERG measurement, with a maximum of 2.5 months, we recorded for each eye: laterality and localization of uveitis, best corrected visual acuity

(BCVA), cell grade in the anterior chamber, cell grade in the vitreous, flare,[11] posterior synechiae, corneal clarity, lens clarity, snowballs, snow banking, vasculitis, optic disc hyperemia or swelling, and CME, either present on OCT (Zeiss, Cirrus HD OCT 5000), or fluorescein angiography (FA).

An experienced ophthalmologist (JdB) scored FAs using the Fluorescein angiographic scoring system of the Angiography Scoring for Uveitis Working Group (ASUWOG).[13] We looked at the FA made before or up to 2.5 months after the ERG was made, for statistical analysis. If more than one FA was made, we used the FA with the highest overall score as an indicator of structural damage of previous severity of inflammation.

We recorded per patient treatment with systemic steroids, methotrexate (MTX), mycophenolate mofetil and adalimumab and we recorded per eye the frequency of administered periocular corticosteroid injections.

Statistical analysis

For statistical analysis SPSS version 21.0 (SPSS Inc., Chicago IL) was used. Elecrroretinography abnormalities were investigated in relation to clinical parameters.

We performed all analyses twice, once using all 63 uveitis eyes, and once using only one eye per patient. We performed this second analysis as an alternative for paired sampling analyses. For this second analysis, we included the eye with the worst BCVA in case of a bilateral uveitis. If BCVA was the same in both eyes, a random eye was selected per patient.

Pearson chi squared test or the likelihood ratio was applied for categorical variables, and a Student t-test or a Mann-Whitney U test was applied for continuous variables. Spearman rank correlation coefficient was used to find possible correlations between variables. We considered p-values below 0.05 as statistically significant. All tests were 2-tailed.

RESULTS

Patient characteristics

Most patients (91%; 30 out of 33) had a bilateral uveitis; 49% of the patients were male. Intermediate uveitis was seen in 64% of the patients and panuveitis in 36% of patients. In 3 patients uveitis was associated with Blau-syndrome, a rare hereditary disorder with a classical triad of arthritis, dermatitis and uveitis associated with a NOD2 mutation.[14] In the other 30 patients no underlying cause was found.

ERG analysis

The ERG was abnormal in 35 (56%) eyes (21 patients, 2 with unilateral uveitis) (Table 1). In line with this, when looking at the specific ERG parameters as continuous variables, uveitis patients did decidedly worse than healthy controls in all cone, 30Hz, rod and combined rod cone responses, with the exception of the rod implicit times (Figure 1 and Supplemental figures 1 - 7).

The most frequently found ERG abnormality (24 eyes (38%)), was a prolonged implicit time of the cone b-wave, which was most pronounced at the lower stimulus strengths (0.3 and 1.0 cds/m^2 LA). In addition, responses showed abnormal waveforms with less steep slopes of both the ascending and descending limb of the b-wave (Figure 2).

The second most frequently found ERG abnormality (21 eyes, 33%) was an abnormal 30 Hz flicker response, consisting of a reduction in amplitude, or a prolonged implicit time, or both (Figure 2).

Table 1: Electroretinogram abnormalities of uveitis eyes (n=63)

		Eyes with ERG abnormalities N (%)
Overall		35 (56)
Single flash cone[a]		27 (43)
A-wave	Amplitude	4 (6)
	Implicit time	2 (3)
B-wave	Amplitude	9 (14)
	Implicit time	24 (38)
30 Hz flicker response[b]		21 (33)
Through	Implicit time	7 (11)
Peak	Amplitude	17 (27)
	Implicit time	12 (19)
Single flash rod[c]		8 (13)
A-wave	Implicit time	2 (3)
B-wave	Amplitude	6 (10)
	Implicit time	0
Single flash combined rod cone[d]		15 (24)
A-wave	Amplitude	11 (18)
	Implicit time	2 (3)
B-wave	Amplitude	7 (11)
	Implicit time	1 2)

Abbreviations: ERG = electroretinography a) light adapted 0.3 - 10.0 cds/m^2; b) light adapted cone response; c) dark adapted 0.0001 - 0.01 cds/m^2; d) dark adapted 3.0 - 30.0 cds/m^2

In 16 eyes (25%) both the implicit times of the cone b-wave were prolonged and the 30 Hz flicker response was abnormal. In 5 eyes the 30 Hz flicker response was abnormal, while the implicit times of the cone b-wave were within normal values, and in 8 eyes the 30 Hz flicker response was within normal values while the implicit times of cone b-waves were prolonged. No statistically significant differences were found in the b/a wave ratios of the cone ERG between uveitis and age matched controls.

Besides abnormalities in the cone b-wave and 30 Hz responses, abnormalities in the rod (0.0001 - 0.01 cds/m^2 DA) and combined rod cone (3.0 - 30.0 cds/m^2 DA) responses were found in 8 eyes (13%) and 15 eyes (24%), respectively. Six eyes (10%) only had abnormalities in the dark-adapted ERG, without ERG abnormalities in the light-adapted ERG.

30 Hz Flicker response

LA b-wave implicit time

Figure 1: Boxplots of the implicit time of the cone b-wave and the 30 Hz flicker response of patients compared to healthy controls from the same age category (5 - 22 years, n = 50). For statistical analysis Mann-Whitney U test was used. Abbreviations: LA = light adapted.
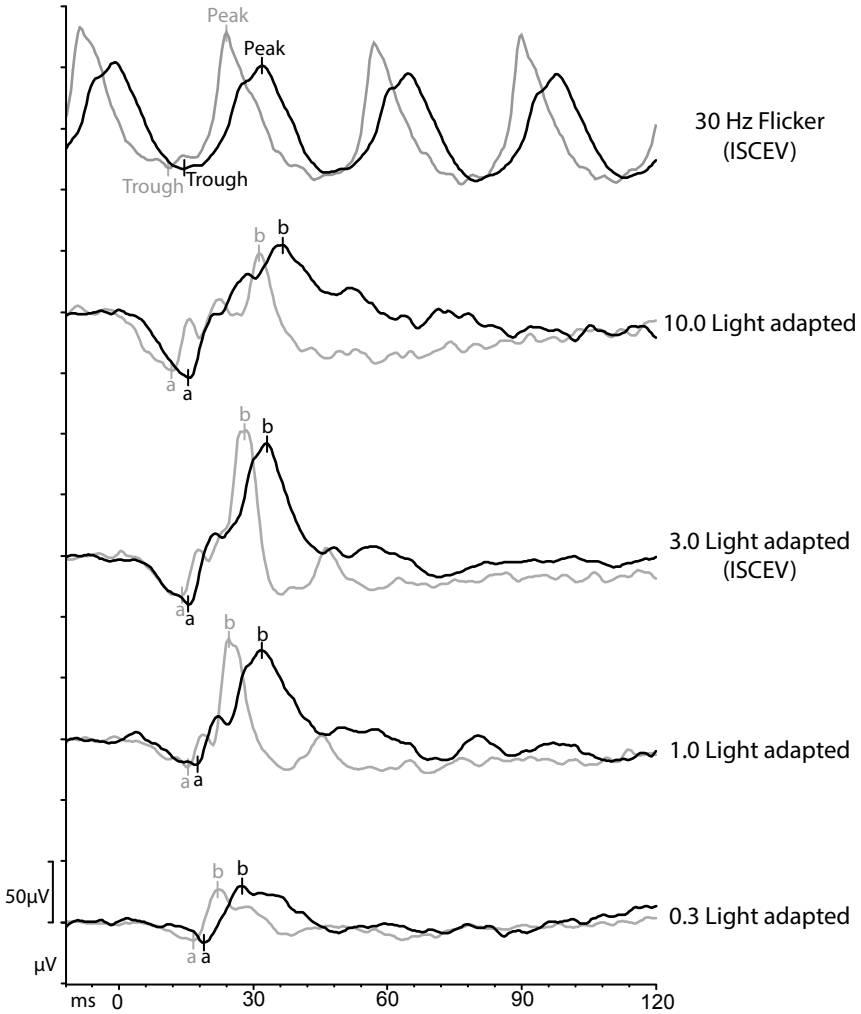
Figure 2: Representative example of a light-adapted (LA) electroretinogram (ERG) of a patient with uveitis (black) and an ERG of a age matched control (grey). The cone b-wave implicit times of the uveitis patient are prolonged; this is most pronounced at the lower stimulus strengths (light adapted 0.3 and 1.0 cds/m²). The 30 Hz flicker response of the uveitis patient is abnormal as well, with reduced amplitudes and prolonged implicit times.

Statistical analysis of ERG abnormalities in relation to clinical parameters

We analyzed if clinical parameters were significantly different between uveitis eyes with and without ERG abnormalities. Here we classified the ERG as abnormal if the implicit time of the cone b-wave was prolonged, or the 30 Hz flicker response was abnormal, or both (Table 2). We used these ERG abnormalities, since they had a typical appearance, occurred frequently and are both cone mediated.

Table 2. Clinical characteristics of uveitis eyes in relation to electroretinogram abnormalities

	Eyes with ERG abnormalities[x] n=29	Eyes without ERG abnormalities[x] n=34	P-value all eyes	p-value worst eye[e]
Male	16 (55)	14 (41)	.267	.565
Age in years[a,b]	11.4 (9.5 -13.2)	14.8 (11.9 - 15.8)	.016*	.067
Duration of uveitis in years[a,b]	1.7 (.7 - 6.4)	3.4 (1.5 - 7.3)	.267	.908
Blau syndrome	1 (3)	5 (15)	.112	.438
History of CME	17 (59)	12 (36)	.080	.309
3+ vitreous cells[b,c]	7 (24)	0	.001**	.026**
3+ cells in anterior chamber[b,c]	2 (7)	0	.075	.266
CME [b]	7 (24)	0	.001**	.021**
Hyperemic optic disc[b]	9 (31)	3 (9)	.023*	.173
Vitreous haze[b,c]	8 (28)	7 (21)	.516	.602
BCVA in LogMAR[a,b]	0.10 (0.00 - 0.40)	0.05 (0.00 - 0.11)	.127	.133
Oral Prednisone	21 (72)	28 (82)	.345	.602
Methotrexate	11 (38)	17 (50)	.337	.246
Adalimumab	5 (17)	7 (21)	.735	.805
Mycofenolaatmofetil	12 (41)	12 (35)	.620	.948
Peribulbar steroid injections	15 (52)	15 (44)	.547	>.999
FA total score[a,d]	11.0 (5.0 -17.0)	8.0 (1.8 - 12.0)	.108	.461
FA capillary leakage score[a,d]	5.0 (0-10.0)	2.0 (0 - 5.3)	.148	.265
FA CME[a,d]	17 (65)	13 (43)	.137	.309
FA vasculitis[a,d]	16 (59)	19 (63)	.752	.466
FA optic disc leakage[a,d]	23 (85)	23 (76)	.413	.391

x) ERG abnormalities are defined as prolonged implicit times of the light adapted (0.3 - 10.0 cds/m²) cone b-wave and/or abnormalities of the 30 Hz flicker (amplitudes/implicit times). Data are given as number (%) unless otherwise stated. a) Data given as median (IQR). b) Present at closest outpatient visit to ERG measurement. c) As described by the Standardization of Uveitis Nomenclature classification. d) FAs with the highest score were used for calculation of FA scores as described by The Angiography Scoring for Uveitis Working Group. e) Only none eye per patient was used, in case of bilateral uveitis, eyes with the worst BCVA were selected, when BCVA was the same, eyes were selected at random. * significant in one of two analysis ** significant in both analyses. Abbreviations: ERG = electroretinography, N = number; BCVA = best corrected visual acuity, LogMAR = logarithm of minimal angle of resolution, CME = cystoid macular edema, FA = fluorescein angiography.

Two clinical parameters were statistically significantly different between eyes with and without these ERG abnormalities in both the analysis using all uveitis eyes, and the analysis using only one eye per patient. These were: CME and 3+ vitreous cells (Table 2). There was no significant correlation between these two variables (Spearman's rho correlation coefficient = 0.259; p = 0.056).

No statistically significant differences were observed between the two groups with regard to treatment, or in the inflammation activity on FA score. Interestingly, we also found no statistical differences in BCVA, which was relatively good in both groups (0.05 and 0.05 LogMAR) (Table 2). Furthermore, no statistically significant differences were observed between clinical parameters and rod and combined rod-cone abnormalities.

DISCUSSION

In this study, more than half of the eyes with non-anterior childhood uveitis showed ERG abnormalities. The light-adapted ERG was most frequently affected, showing a prolonged cone b-wave implicit time, particularly at lower stimulus strengths, and abnormal 30 Hz flicker responses. Two clinical parameters were statistically significantly associated with these ERG abnormalities: CME and 3+ vitreous cells.

All eyes with CME had abnormal cone ERGs. Although cones are densely packed in the macula,[15,16] macular dysfunction alone contributes only minimally to the full field cone.[17–19] The abnormal ERG in the children with CME therefore indicates a global retinal dysfunction and not only macular dysfunction.[18]

A correlation between visual acuity and outer retinal function (represented by the a-wave) has been described, which is in line with our study in which we found a relatively good visual acuity and few a-wave abnormalities.[20,21] However, a correlation between visual acuity in inner retinal function (represented by the b-wave) as found in birdshot uveitis, was not confirmed in our study on childhood uveitis.[22] This discrepancy between birdshot and childhood uveitis may be due to a disease-specific inflammatory mechanism or to the duration of disease.

Besides eyes with CME, all eyes with 3+ vitreous cells also showed ERG abnormalities, indicating that more severe inflammation frequently results in retinal dysfunction.

The prolonged b-wave implicit time and the abnormal 30 Hz flicker response indicate an abnormal inner retinal transmission dysfunction from photoreceptors to bipolar cells.[18] This abnormal signal transmission could be caused by an increased interneuronal distance secondary to an increased permeability of the blood-retinal

barrier, caused by inflammation.[23] Although the prolonged cone b-wave and the 30 Hz flicker response indicate inner retinal dysfunction, amplitudes were mostly normal, as were b/a wave ratios. In addition to the abnormal cone ERGs, we also found some abnormalities in the dark-adapted ERG. Although it may be interesting to investigate these abnormalities in more detail, in this study we decided to focus on the more profound and more frequent abnormalities of the cone ERG. Here we saw a consistent and recognizable pattern in the prolonged cone b-wave implicit time and the abnormal 30 Hz flicker response.

Our study is the first one that describes ERG changes in childhood uveitis by using an extended ISCEV based protocol, with a greater range of stimulus strengths than the ISCEV standard protocol. The abnormal timing in the cone b-wave, which we found in our study, was most profound at lower stimulus strengths (0.3 cds/m^2 LA and 1.0 cds/m^2 LA), and therefore may not have been discovered by using the standard protocol only.

Previous reports on intermediate uveitis and childhood uveitis mostly describe abnormalities in amplitudes but rarely describe implicit times.[24–26] Shamshinova et al. found a subnormal ERG response in 75% of eyes in childhood uveitis, including all anatomic subtypes. ERG abnormalities were more frequently seen when the macula was affected and in non-anterior uveitis.[9]

In accordance with our findings, abnormalities of the 30 Hz flicker response in intermediate uveitis have been described. However, abnormal implicit times of the combined rod-cone response have also been reported, whereas we mostly found abnormal cone b-wave implicit times.[24] Other studies on intermediate uveitis mainly describe differences in ERG amplitudes and do not mention implicit times.[25,26]

There are several limitations to this study. Due to the retrospective design and limited sample size, weak associations may not have been found. Since pediatric uveitis is not a common entity, we were unable to include more patients. Additionally, we were unable to correct for paired eyes, which would have been preferable since most patients had a bilateral uveitis. We were unable to perform a generalized estimating equation (GEE), since we had a complete separation of data in multiple variables, including CME and the amount of vitreous cells. By using only one eye per patient, we would have discarded almost half of the limited amount of data. Therefore, we decided to perform and present both the analysis with all eyes, and the analysis with only one eye per patient.

We did not find statistically significant associations with ERG abnormalities and FA scores in both analyses. We also could not correlate ERG abnormalities to visual field defects, as visual fields were only assessed in a minority of children and were often made a long time before the ERG was performed.

Considering the current findings and previous reports, we emphasize that in non-anterior childhood uveitis the global inner retinal function is frequently affected. Even though ERG abnormalities in intermediate and childhood uveitis have been reported in the past, ERG outcomes were not investigated in relation to clinical parameters as shown in our study. We recommend using an extended ISCEV protocol to detect early and subtle retinal dysfunction. If an association between ERG abnormalities and long-term visual outcome can be made in the future, these early ERG findings during the course of childhood uveitis have significance for treatment strategies. Since ERG abnormalities occur when BCVA is still relatively good, further studies should focus on the effects of this retinal dysfunction on long term visual outcome.

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SUPPLEMENTAL MATERIAL
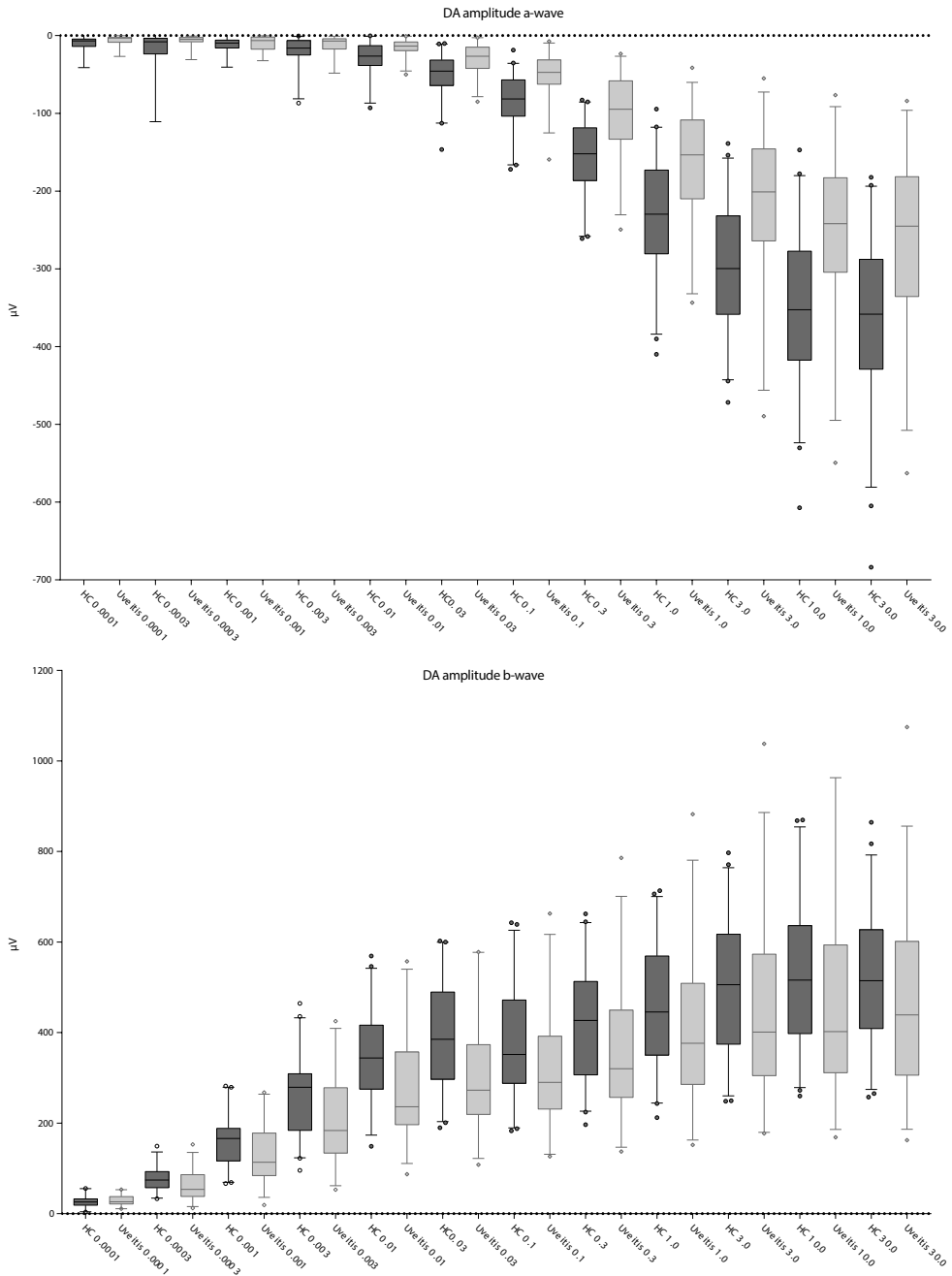
Supplemental figures 1 (top) and 2 (bottom): Boxplots of the amplitude of the rod and combined rod-cone a-wave (top) and b-wave (bottom) of patients compared to healthy controls from the same age category (5 - 22 years, n = 50). Uveitis patients has statistically significant worse outcomes for rod and combined rod-cone responses compared to healthy controls using Mann-Whitney U test. Abbreviations: DA = dark adapted, HC = healthy control.

DA implicit time a-wave

DA implicit time b-wave

Supplemental figures 3 (top) and 4 (bottom): Boxplots of the implicit times of the rod and combined rod-cone a-wave (top) and b-wave (bottom) of patients compared to healthy controls from the same age category (5 - 22 years, n = 50). Uveitis patients has statistically significant worse outcomes for the combined rod-cone response compared to healthy controls using Mann-Whitney U test. Abbreviations: DA = dark adapted, HC = healthy control.
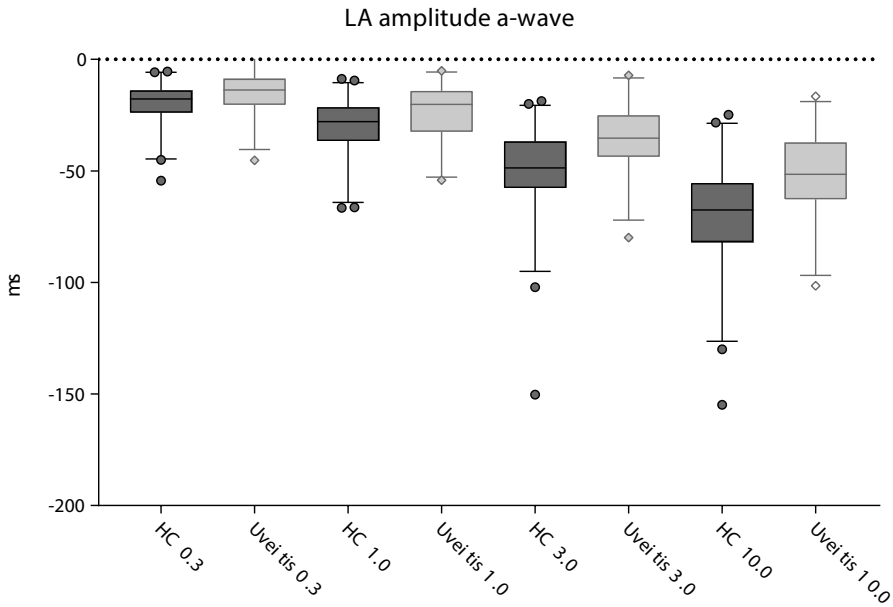
LA amplitude a-wave

Supplemental figure 5: Boxplots of the amplitude of the cone a-wave of patients compared to healthy controls from the same age category (5 - 22 years, n = 50). Uveitis patients has statistically significant worse outcomes in all flash intensities compared to healthy controls using Mann-Whitney U test. Abbreviations: LA = light adapted, HC = healthy control.
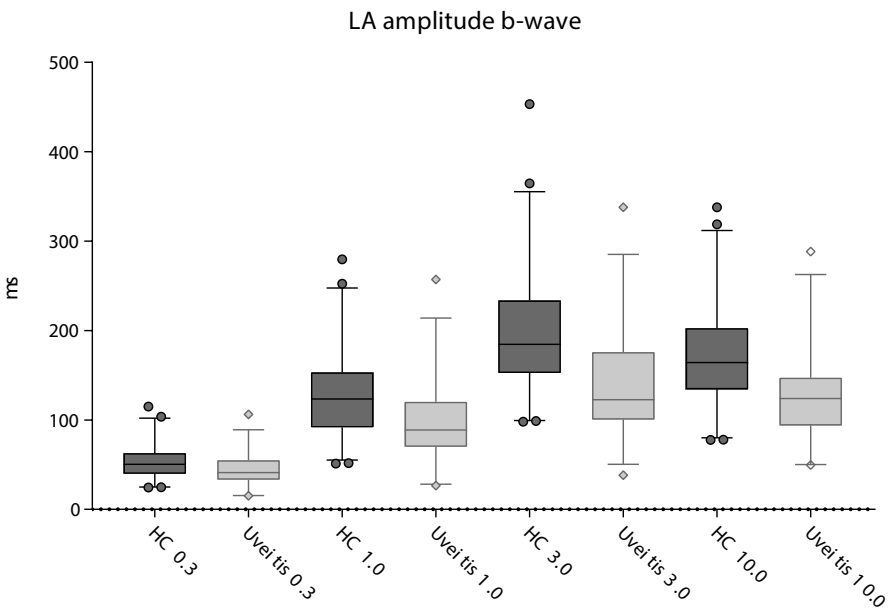
LA amplitude b-wave

Supplemental figure 6: Boxplots of the amplitude of the cone b-wave of patients compared to healthy controls from the same age category (5 - 22 years, n = 50). Uveitis patients has statistically significant worse outcomes in all flash intensities compared to healthy controls using Mann-Whitney U test. Abbreviations: LA = light adapted, HC = healthy control.
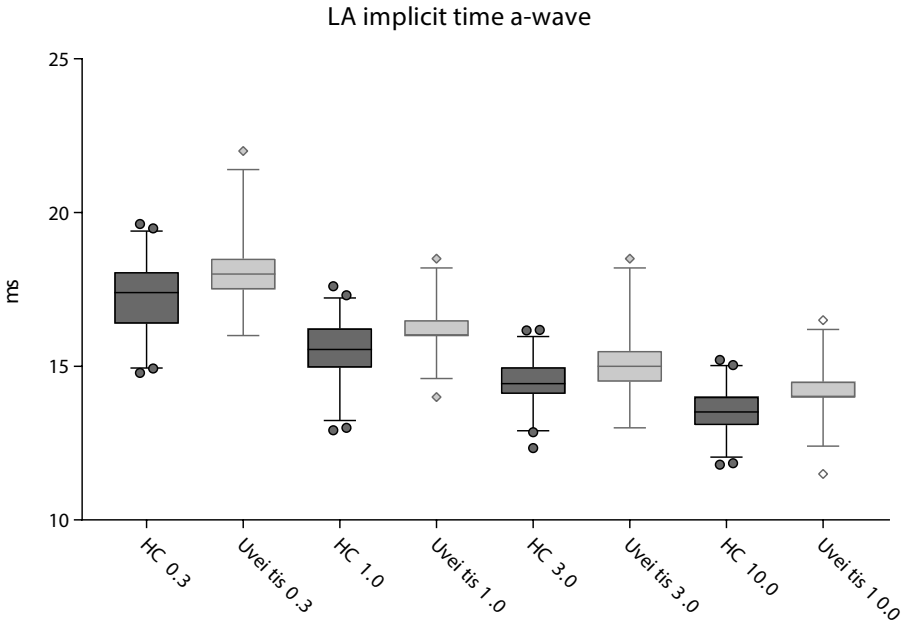
Supplemental figure 7: Boxplots of the implicit time of the cone a-wave of patients compared to healthy controls from the same age category (5 - 22 years, n=50). Uveitis patients has statistically significant worse outcomes in all flash intensities compared to healthy controls using Mann-Whitney U test. Abbreviations: LA = light adapted, HC = healthy control.
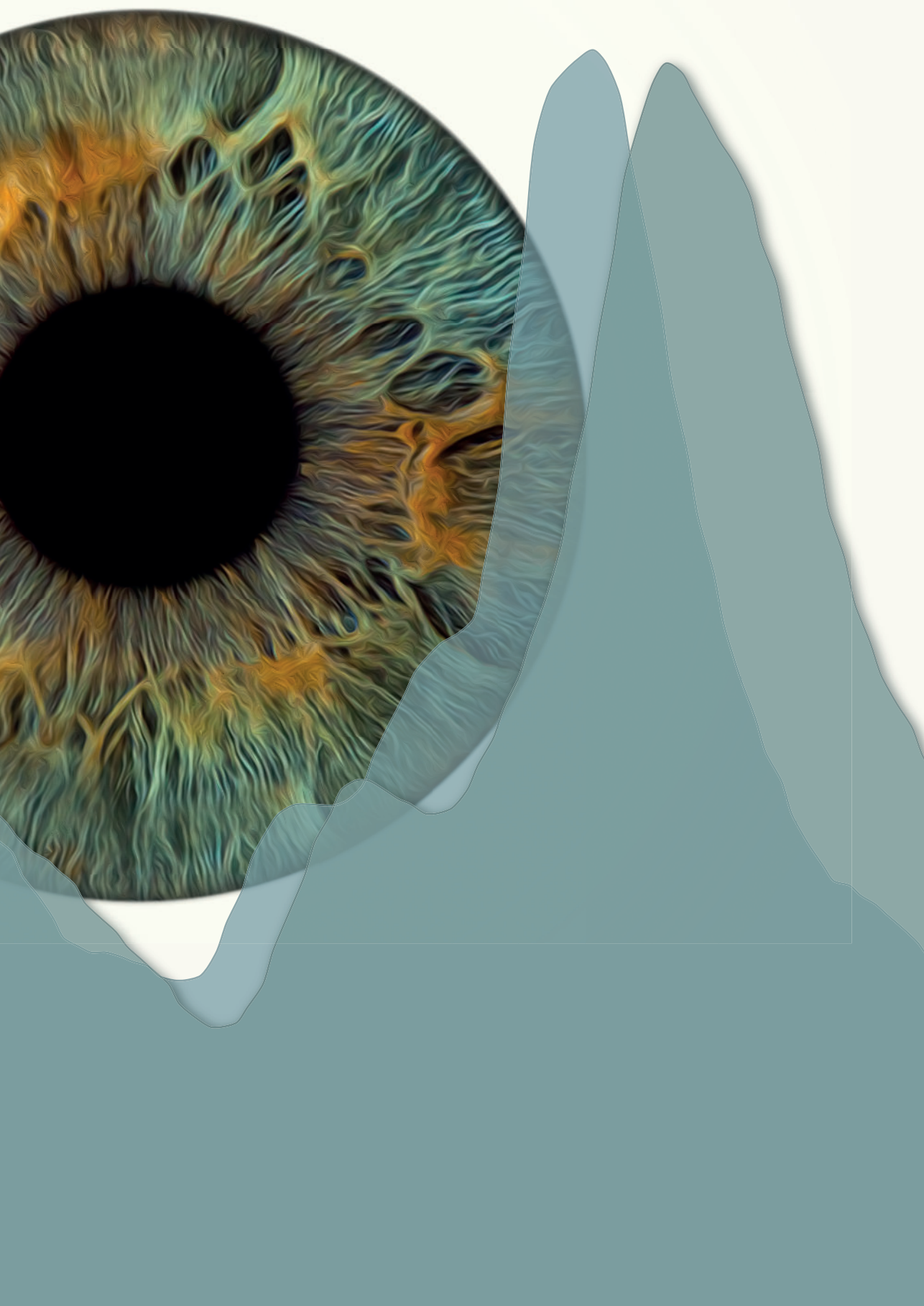
CHAPTER 6

JUVENILE IDIOPATHIC ARTHRITIS UVEITIS: A TYPICAL ANTERIOR UVEITIS, BUT THE RETINAL FUNCTION CAN BE AFFECTED

Anna H Brouwer
Joke H de Boer

ABSTRACT

Purpose

To describe the retinal function as measured by full-field electroretinogram (ERG) in patients with juvenile idiopathic arthritis (JIA) associated uveitis.

Methods

ERGs were measured according to an extended ISCEV protocol.

Results

Ten of the 16 uveitis eyes (8 patients) showed ERG abnormalities. The type of ERG abnormalities varied amongst patients and occurred in both amplitudes and implicit times, in a-waves, b-waves, 30 Hz flicker response and in both the dark adapted and the light adapted ERG. Five eyes had an active inflammation at the time of the ERG recording; all of these showed ERG abnormalities. Five out of eleven inactive uveitis eyes showed ERG abnormalities. No correlations were found between ERG abnormalities and the duration of uveitis, mean deviation on visual field analysis, or central macular thickness. None of the eyes had severe media opacities which could have explained the observed ERG abnormalities.

Conclusion

Even though JIA is a typical anterior uveitis, the retinal function can be affected in this type of uveitis.

Dear editor,

Juvenile idiopathic arthritis (JIA) associated uveitis is typically described as a chronic anterior uveitis. We would like to present 8 patients with longstanding JIA uveitis (aged between 17 - 40 years) with suspicional retinal thinning on optical coherence tomography (OCT), of whom we additionally measured a full-field electroretinogram (ERG). In 10 of the 16 uveitis eyes we observed ERG abnormalities (Figure 1). These ERG abnormalities indicate that although the inflammation in JIA is mainly located in the anterior part of the eye, the retina can be affected as well. To the best of our knowledge, this is the first report that describes that the ERG can be affected in this particular type of uveitis.

The ERGs were measured according to an extended international society for clinical electrophysiology of vision (ISCEV) protocol. This protocol incorporates the ISCEV standards, but includes more flash strengths than the standard ISCEV protocol. The flash strengths increase with approximately 0.5 log units steps and range from 0.0001 - 30.0 cds/m^2 (12 flash strengths) for the dark-adapted ERG (DA) and from 0.3 - 10.0 cds/m^2 (4 flash strengths) for the light-adapted ERG (LA) and include a 30 Hz flicker response. Dawson-Trick-Litzkow (DTL) electrodes were used as corneal active electrodes, and an Espion E3 system with Colordome stimulator (Diagnosys LLC, Cambridge, UK) for flash stimulation. Our reference values were previously described.[1]

The type of ERG abnormalities varied among patients. Table 1 gives an overview of the ERG results. We observed abnormalities in all parts of the ERG: both in amplitudes and implicit times, in a-waves, b-waves and the 30 Hz flicker response. Abnormalities occurred in both the dark adapted (DA) and the light adapted (LA) ERG. The only ERG parameter which showed no abnormalities was the implicit time of the rod b-wave indicating that the rod response might be less affected than the cone response in JIA uveitis. In some eyes the amplitudes were severely diminished, whereas in others the implicit times were mainly prolonged. The most frequent ERG abnormality was a prolonged cone a-wave, which was abnormal in eight eyes. These findings highlight that different aspects of retinal function can be affected in JIA uveitis, which is in line with other forms of uveitis where ERG abnormalities may be observed in all aspects of the ERG, but the light adapted ERG is most frequently affected.[1]

We previously reported a characteristic type of ERG abnormality in non-infectious uveitis: a prolonged cone b-wave, which was associated with the severity of inflammation, but in these studies no patients with JIA uveitis were included.[1,2] The ERGs of the current JIA patients showed a prolonged cone b-wave in 6 eyes. Recently we observed that the prolonged cone b-wave can improve in other forms of uveitis, particularly in eyes where the inflammation in the anterior chamber becomes less

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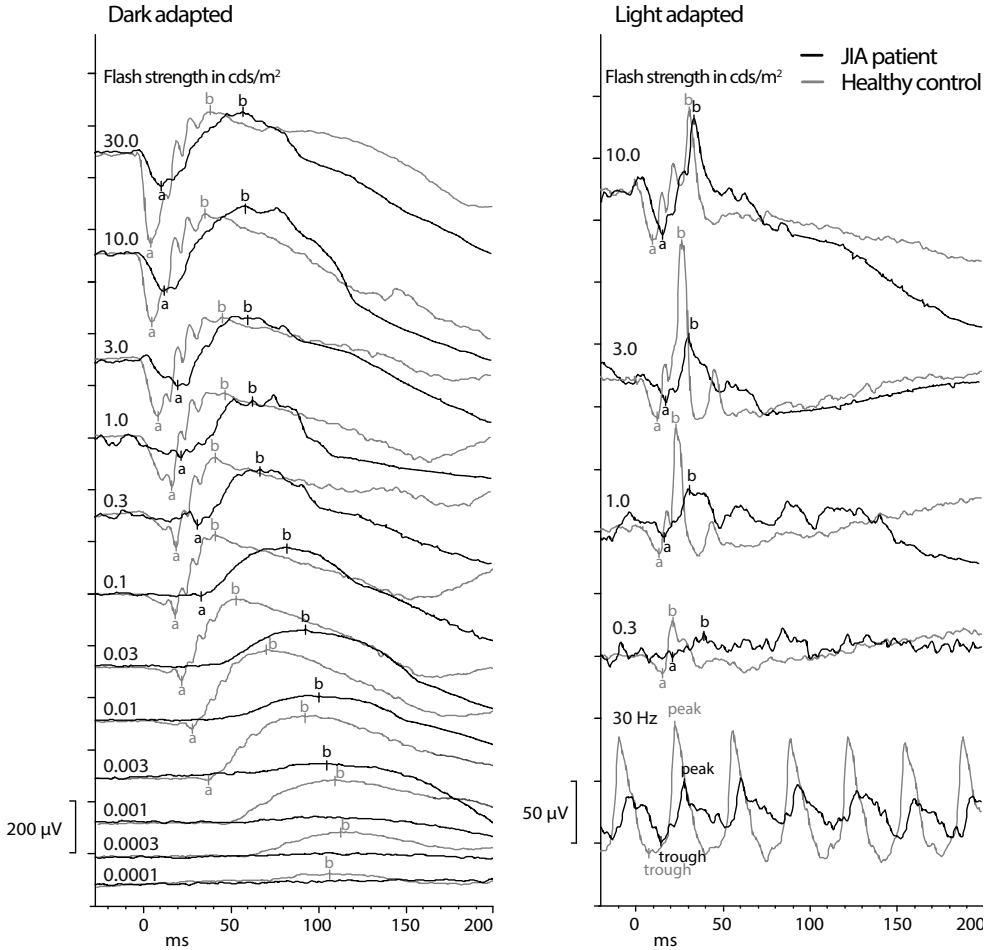
Example of ERG curves of a JIA uveitis patient (black) and an ERG of a healthy control (grey). The implicit times of a-waves and b-waves of both the dark adapted and light adapted are prolonged. Amplitudes are often reduced as well. Abbreviations: ERG = electroretinogram, JIA = juvenile idiopathic arthritis, cds/m² = candela · seconds/squared meters, ms = milliseconds, µV = microvolts.

active.[3] This might indicate that the observed ERG abnormalities in these JIA patients can also improve.

However, we have also observed that ERGs of uveitis patients can deteriorate over time.[3] If this can also occur in JIA uveitis, should be the subject of further studies. There are reports that retinal atrophy can occur in JIA patients, usually as the result of chronic cystoid macular edema (CME), a complication of JIA uveitis.[4,5] None of the JIA eyes had a retinal atrophy (central macular thickness (CMT) < 150 µm), but because ERG abnormalities may occur prior to retinal atrophy, we investigated the effects of CME on the ERG. We found no association between CMT and ERG abnormalities (Spearman's correlation coefficient -.267, p = 0.317). At the time of the ERG recording,

Table 1: Overview of electroretinogram abnormalities and clinical parameters of patients with juvenile idiopathic arthritis associated uveitis

case	eye	LA cone ERG a-wave amp	LA cone ERG a-wave IT	LA cone ERG b-wave amp	LA cone ERG b-wave lat	LA cone ERG 30 Hz amp	LA cone ERG 30 Hz lat	DA rod b-wave amp	DA rod b-wave lat	DA rod/cone a-wave amp	DA rod/cone a-wave lat	DA rod/cone b-wave amp	DA rod/cone b-wave lat	OVERALL ERG response	Active[a]	Vision[b]	Systemic treatment[c]
case 1	OD		↑		↑		↑							X	yes	0,22	yes
	OS	↓	↑	→	↑	→	↑							X	no	1,00	yes
case 2	OD		↑	→		→		→		→	↑	→		√	no	0,05	no
	OS					→								X	yes	0,10	no
case 3	OD		↑	→		→		→		→		→		X	no	0,05	yes
	OS													√	no	0,00	yes
case 4	OD		↑		↑		↑			→	↑		↑	X	no	0,00	yes
	OS		↑		↑						↑		↑	X	no	0,00	yes
case 5	OD	↓	↑											√	no	-0,08	yes
	OS													X	no	-0,08	yes
case 6	OD		↑	→	↑	→		→		→		→		√	no	0,00	no
	OS			→		→		→		→	↑		↑	X	no	0,30	no
case 7	OD							→		→		→		√	no	0,00	yes
	OS						↑				↑		↑	X	yes	0,05	yes
case 8	OD		↑											√	no	-0,05	no
	OS													X	yes	0,00	no

Overview of electroretinogram results of eight patients with juvenile idiopathic arthritis associated uveitis. ERGs were recorded according to an extended protocol which incorporates ISCEV standards. ↓ = amplitude reduced; below 5th percentile of reference values → = implicit time prolonged; above 95th percentile of reference values √ = ERG showed no abnormalities X = ERG showed some abnormalities a) Eyes with activity had in these cases either cells in the anterior chamber, or flare or both. b) in LogMAR c) All patients with systemic treatment in these cases were treated with adalimumab and methotrexate. Abbreviations: ERG = electroretinogram, LA = light adapted, DA = dark adapted, Amp = amplitude, IT = implicit time, RE = right eye, LE = left eye.

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the median CMT of all eyes was 264 µm (IQR 250 - 260). Three eyes of the sixteen eyes had diffuse CME (CMT) > 304 µm on optical coherence tomography (OCT) at some point during their follow-up. Two eyes had a longer period of hypotony which resulted in macular folds. These folds were still present at the day of the ERG.

One patient had a history of a retinal detachment in his left eye (case 1) which was treated with a posterior vitrectomy with silicon oil. The oil was removed 5 years prior to the ERG recording. The ERGs of this patient were severely abnormal in both eyes, but as expected, the eye with a history of retinal detachment was more affected.

We did not find an association between a type of treatment and the absence or presence of ERG abnormalities. Five patients were treated with systemic medication (adalimumab and methotrexate) at the time of ERG recording and two patients were treated with only ocular steroid droplets. One patient had no medication at the time of ERG recording. This patient showed no signs of inflammation, but had an abnormal ERG in one eye, possibly due to the effects of end-stage glaucoma. These findings are in line with our previous studies were we also found no association between type of treatment and ERG abnormalities.[1–3] The absence of such an association could be due to the small population, different treatment strategies, and heterogeneity in the population due to responders and non-responders to treatment.

In our previous studies we found a strong association between an active inflammation and ERG abnormalities.[1,2] In the current series, five eyes showed some signs of active inflammation (trace of anterior chamber cells, flare range 1+ - 3+) at the time of ERG recording and all of them had an abnormal ERG. However, of the eleven eyes that had an inactive uveitis at the time of the ERG recording, five had an abnormal ERG.

Although ERG abnormalities can occur early in the disease process of uveitis,[1] in the current series the disease duration was long (median 18.9 years, IQR 16.6 - 19.8). If ERG abnormalities can also be observed in patients with a short duration of uveitis in JIA, remains therefore speculative. However, we found no association between the duration of JIA uveitis and ERG abnormalities (Spearman's correlation coefficient 1.0, p > .999). Also, no relation was found between the mean deviation on VF and ERG abnormalities (median -3.14, IQR -4.65 - -0.80, Spearman's correlation coefficient -.392 p = 0.143).

None of the patients had severe media opacities that could have influenced the ERG results. All pupils were fully dilated at the time of ERG recording and none of the eyes had posterior synechiae at the time of ERG recording. None of the patients had central band keratopathy.

With this letter we hope to illustrate to electro-physicists and ophthalmologists treating JIA uveitis that the retinal function can be affected in JIA uveitis, even though

the primary site of inflammation in this form of uveitis is in the anterior part of the eye. Further research is needed to evaluate if there is, similar to other forms of uveitis, an associations between the activity of uveitis and ERG abnormalities in JIA uveitis. If such an association is found, ERG abnormalities could warrant an intensifying of treatment in JIA uveitis in order to prevent a permanent loss of retinal function.

ACKNOWLEDGEMENTS

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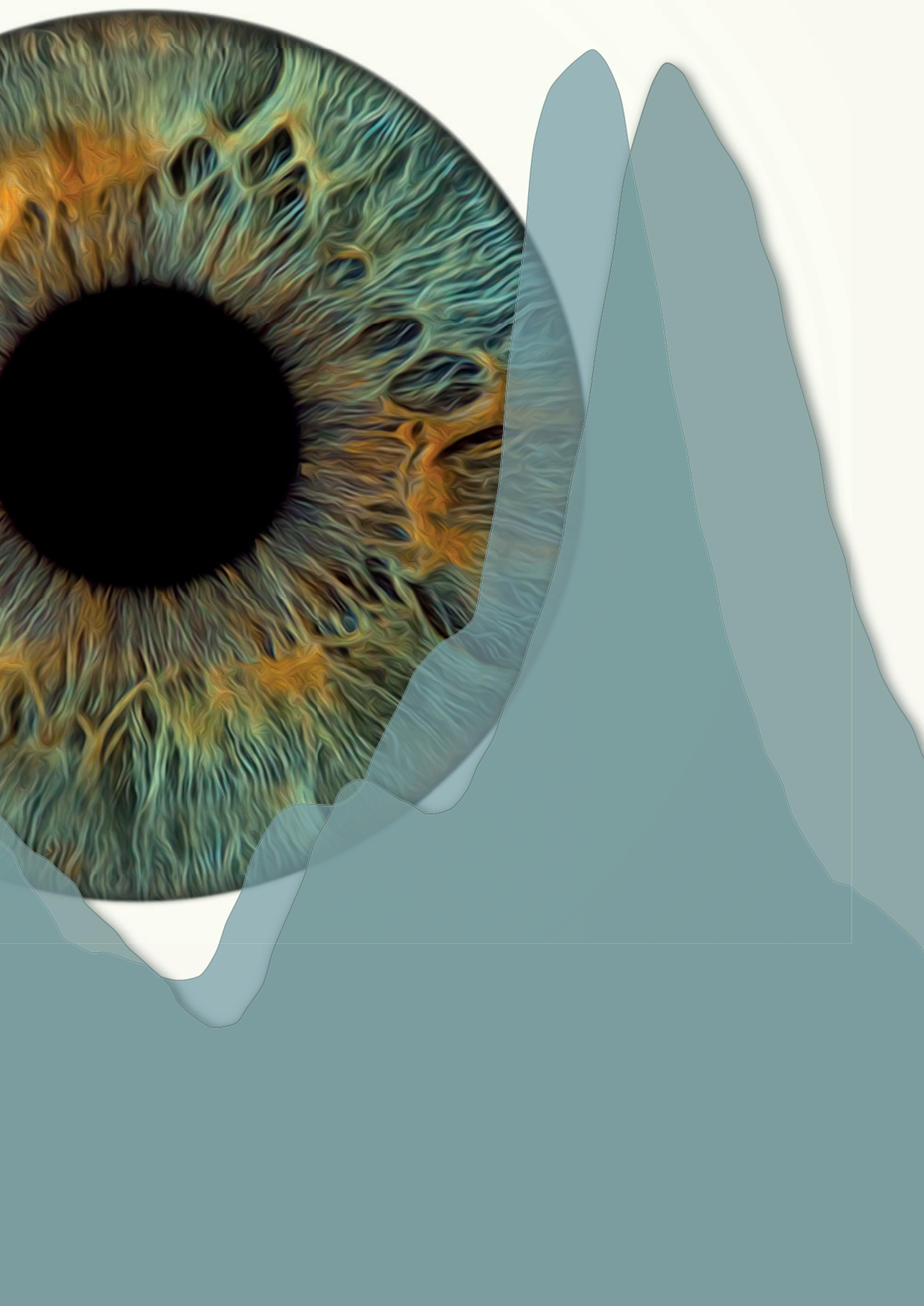
CHAPTER 7

EFFECTS OF DTL ELECTRODE POSITION ON THE AMPLITUDE AND IMPLICIT TIME OF THE ELECTRORETINOGRAM

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ABSTRACT

Purpose

This study sought to investigate whether there is an optimal position of the Dawson, Trick, and Litzkow (DTL) electrodes when measuring the full-field electroretinogram (ERG) for monitoring purposes.

Methods

In 200 uveitis patients an extended light adapted (LA) ERG protocol was measured twice, incorporating the International Society for Clinical Electrophysiology of Vision standards. First a LA ERG was measured with the DTL in the lower lid position (LLP), and thereafter in the fornix position. Differences in amplitudes and implicit times of a-waves, b-waves and the 30 Hz peak were investigated. Intraclass correlation coefficients (ICC) as well as coefficients of variation (CoV) were calculated, to assess both reliability and relative variability between the two DTL positions.

Results

Implicit times showed no statistically significant differences between the two DTL positions. As expected, amplitudes at the different stimulus strengths were 1.12 - 1.19 higher in the LLP, but there were no significant differences in the CoV between the two DTL positions. The ICC was high for the b-wave and 30 Hz flicker response (0.842 - 0.979), but lower for the a-wave, especially for amplitudes (0.584 - 0.716).

Conclusions

For monitoring purposes in patients, we conclude that based on relative variability, no position is preferable above the other. However, because in most diseases amplitudes are decreased, the LLP may be chosen because it yields higher amplitudes. Whatever the choice, it is important to ensure that the DTL position remains stable during an ERG recording.

INTRODUCTION

Since the introduction of the Dawson Trick Litzkov (DTL) electrode[1], its use in recording electroretinograms (ERGs) has spread. One of the main advantages of the DTL is that it is much more comfortable to wear compared to conventional electrodes, such as contact lens electrodes.[2–4]

Originally, the ERG was mainly used for diagnosing retinal diseases such as retinal dystrophies, where the ERG is frequently severely abnormal. However, now that the ERG is increasingly used for monitoring disease, more subtle ERG changes become important. Therefore, one must be aware of factors that may affect the ERG results, other than disease or treatment. Particularly factors that influence the inter-session variability are important.

Factors that may influence the absolute ERG results, but will have little effect on inter-session variability since they do not differ between sessions, include gender,[5] refraction,[6] and ocular pigmentation.[7] Other factors that can affect the intersession-variability may be minimized by always incorporating International Society for Clinical Electrophysiology of Vision (ISCEV) standards. These include duration of dark- and light adaptation, flash strengths and pupil size.[8] Besides these, there are factors that also affect the intersession variability but are more difficult to address. These include: DTL position,[9–12] media opacities,[13] and age.[14–16]

When the DTL electrode was introduced, it was said that its position was 'only a little disturbed by blinking'.[1] However, since then several reports state that the DTL position can shift, which may significantly affect the recorded amplitudes.

When the DTL is positioned on the cornea, the highest amplitudes can be recorded, which decrease as the DTL is positioned more toward the fornix.[17] Despite these differences in amplitudes, the latest ISCEV standard does not advice which DTL position should be used. The most common DTL positions are: the lower lid position (LLP) and the fornix position (FP).[8]

In our clinic, reference values are based on ERG measurements recorded at the LLP. This position was advised by the manufacturer for two reasons. First, it would ensure high amplitudes, without much risk of scratching the cornea. Second, blinking would have little effect on the DTL position. In some cases however, we have seen the DTL shift toward the fornix during an ERG recording.

Some studies advise using the FP, because it would shift less easily and therefore lead to a more stable recording. The FP would yield lower amplitudes but the ERG would be less variable.[9–11] However, these studies were conducted in relatively small groups of healthy volunteers. These healthy volunteers are probably often coworkers who know how to cooperate during an ERG since they are familiar with ERG

procedures. Therefore, it is useful to see if the same results can be obtained in a large cohort of patients, who may be scared or photophobic which makes the recording of an ERG more difficult. Also, a larger group can yield more reliable confidence intervals of differences between the two DTL positions.

The aim of this study was to determine the optimal DTL electrode position for monitoring purposes. We investigated whether one DTL position yields more stable ERG results than the other. If such a difference is found, this would imply that this DTL electrode position should be used for monitoring purposes. We compared the reliability as well as the relative variability of the results and investigated differences in amplitudes and implicit times.

METHODS

Subjects

The subject population, consisting of 200 patients (355 uveitis eyes, 45 unaffected eyes) with a non-infectious uveitis, aged ≥ 18 years (median 53.4, IQR 39.2 - 63.7), has been previously described.[18] All patients were mentally competent and gave their written consent to participate. This study was conducted in compliance with the ethical principles of the declaration of Helsinki. Ethical approval was requested and obtained from the Medical Ethical Research Committee of the University Medical Centre Utrecht.

ERG measurement

All ERGs were measured according to the ISCEV standards.[8] An Espion E3 system with Colordome stimulator (Diagnosys LLC, Cambridge, UK) was used for full-field flash stimulation. Eyes were anesthetized with oxybuprocain 0.4%. Pupils were dilated with tropicamide 0.5%. Cup electrodes were used as ground and reference electrodes and placed on the forehead and on the temples. Impedances of reference and DTL electrodes were below 5 kΩ and below 10kΩ for the ground electrode.
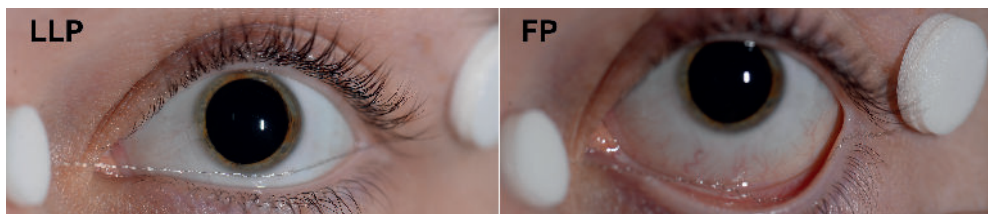
Figure 1: Representative example of the two Dawson, Trick, Litzkov (DTL) positions that were used: the lower lid position (LLP) (left) and the fornix position (FP) (right).

An extended ISCEV protocol was measured twice, consisting of stimulus strengths that increase with approximately 0.5 log units and range from 0.3 to 10.0 cds/m^2 for the light-adapted ERG (LA), including a 30 Hz flicker response (LA, 3.0 cds/m^2). For analyses, averages were used of two results, each consisting of five sweeps. In cases when sweeps with a trend were accepted during recording, these sweeps were toggled, or removed.

After 10 minutes of light adaptation, the first LA ERG was recorded with the DTL electrode in the LLP, and the second with the FP (see Figure 1). The DTL position was checked prior to each measurement and adjusted if necessary.

Analyses

For statistical analysis R. Studio version 1.0.143 was used. To compare differences between the two DTL positions, a Wilcoxon signed rank test was used (data not normally distributed as tested with histograms, Q-Q-plots and Shapiro-Wilk test).

We added a random value between -0.25 and 0.25 ms to each implicit time, because our equipment measures every 0.5 ms. By adding this random value, the implicit time becomes a true continuous variable which has statistical advantages for calculating more reliable confidence intervals. The implicit time difference and amplitude ratio of the two DTL positions were calculated and plotted.

To evaluate the effect of the two DTL positions on the percentage of patients that would fall outside normal limits, we compared the results to our reference values. These reference values were obtained with the DTL in LLP and were previously described.[18]

To investigate the amount of reliability between the two measurements, intraclass correlation coefficients (ICCs) were calculated (two-way model, type consistency, unit average). For visualization of the amount of agreement between the two positions, Bland-Altman plots were made as well.

To evaluate the amount of relative variability of the ERG data at the two DTL positions, we calculated the coefficients of variation (CoV, sd/mean), also known as relative standard deviation. The data were transformed (square root) to fit a normal distribution. This was necessary since the results of uveitis patients can range from normal to abnormal which gives a skewed distribution.[18] Normality was evaluated using Shapiro Wilk tests, histograms and Q-Q-plots. We used the R package cvequality (Version 0.1.3; Marwick and Krishnamoorthy 2018) to test for significant differences in CoV. Using Bonferroni's correction, we defined p-values of < 0.006 as statistically significant. All significances were two-tailed.

RESULTS

Table 1 shows the results of the ERG responses of the different DTL positions. Median amplitudes were significantly higher (between 1.12 and 1.19 for the different stimulus strengths, see Table 2) and amplitude ranges were wider for all responses recorded with LLP compared to the FP. In contrast, there were no significant differences in implicit times between the two DTL positions. Figure 2 shows a representative example of ERGs curves of both DTL positions obtained from the same patient.

To highlight what the effects may be of an unnoticed shift in DTL position on ERG results, we compared the results of the LLP and the FP to our reference values (obtained with the DTL in the LLP). Here, amplitudes were much more frequently

Table 1. ERG results and differences of two DTL electrode positions

	Lower lid		Fornix		
	median	[IQR]	median	[IQR]	p-value
A-wave amplitude*					
0.3	-11.8	[-15.5, -8.1]	-10.1	[-13.0, -7.4]	<0.001***
1.0	-19.4	[-25.1, -14.3]	-16.3	[-20.1, -11.9]	<0.001***
3.0	-29.7	[-37.8, -22.2]	-24.9	[-30.5, -19.8]	<0.001***
10.0	-46.1	[-57.2, -35.4]	-37.4	[-45.9, -31.4]	<0.001***
A-wave implicit time*					
0.3	19.0	[18.2, 20.0]	18.9	[18.0, 20.1]	0.445
1.0	17.1	[16.4, 18.0]	17.1	[16.3, 17.8]	0.115
3.0	15.7	[15.1, 16.4]	15.8	[15.1, 16.5]	0.816
10.0	14.8	[14.3, 15.5]	14.8	[14.2, 15.5]	0.119
B-wave amplitude*					
0.3	33.3	[23.3, 42.0]	26.2	[20.4, 34.6]	<0.001***
1.0	76.0	[55.5, 97.6]	63.1	[49.9, 82.4]	<0.001***
3.0	119.8	[92.2, 151.7]	103.2	[81.7, 129.1]	<0.001***
10.0	118.4	[93.0, 144.6]	105.0	[82.6, 122.8]	<0.001***
B-wave implicit time*					
0.3	27.0	[25.8, 30.0]	27.2	[25.6, 29.9]	0.718
1.0	28.9	[27.8, 31.0]	28.8	[27.7, 30.8]	0.105
3.0	31.6	[30.7, 33.0]	31.6	[30.7, 33.0]	0.129
10.0	35.9	[34.8, 37.3]	36.0	[34.7, 37.2]	0.055
30 Hz flicker Peak					
Amplitude	67.1	[47.2, 86.3]	58.0	[43.9, 74.7]	<0.001***
Implicit time	29.1	[27.5, 31.3]	29.1	[27.6, 31.3]	0.461

*Descriptive statistics of the ERG responses of the two DTL positions (LLP and FP). * Results of different flash strengths in candela · seconds/meters squared of the light adapted ERG.*** statistically significant Abbreviations: ERG = electroretinogram, DTL = Dawson, Trick, Litzkov electrode, IQR = inter quartile range, LLP = lower lid position, FP = fornix position.*

Table 2. Amplitude ratio and implicit time difference of the two DTL positions

Amplitude ratio		Median	[IQR]	Percentiles 2.5th	97.5th
A-wave	0.3*	1.18	[0.85, 1.56]	0.35	3.73
	1.0*	1.18	[0.93, 1.52]	0.55	3.06
	3.0*	1.19	[0.98, 1.43]	0.55	2.34
	10.0*	1.19	[1.00, 1.44]	0.71	2.11
B-wave	0.3*	1.19	[1.01, 1.39]	0.73	2.23
	1.0*	1.13	[1.00, 1.33]	0.79	1.80
	3.0*	1.15	[1.00, 1.33]	0.78	1.75
	10.0*	1.12	[1.00, 1.31]	0.76	1.79
30 Hz flicker response		1.12	[0.97, 1.31]	0.73	1.80

Implicit time difference		Mean	(SD)	Percentiles 2.5th	97.5th
A-wave	0.3*	0.03	(1.32)	-3.22	2.47
	1.0*	0.07	(0.92)	-1.75	1.69
	3.0*	-0.02	(0.79)	-2.18	1.50
	10.0*	0.05	(0.72)	-1.41	1.31
B-wave	0.3*	-0.03	(1.36)	-2.85	2.93
	1.0*	0.02	(0.85)	-1.71	1.64
	3.0*	0.04	(0.80)	-1.92	1.90
	10.0*	0.05	(0.63)	-1.61	1.48
30 Hz flicker response		0.01	(0.85)	-1.73	1.70

*Results of the amplitude ratio (lower lid position/ fornix position) and implicit time difference (lower lid position-fornix position) of the different flash strengths in candela · seconds/meters squared of the light adapted ERG. *candela · seconds/meters squared.*

defined as abnormal when the FP was used (Supplemental table 1). However, in 5% (20 eyes) the ERG was defined as abnormal in the LLP, but normal in the FP.

Figure 3 shows the amplitude ratio LLP/FP and the implicit time difference LLP - FP, for the b-wave (3.0 cds/m^2) of both uveitis eyes (circle) and unaffected eyes (square). It illustrates that in most cases the amplitude ratio is higher than 1.0 which implies that the amplitude that was measured at the LLP was higher than at the FP. In contrast to this, the implicit time difference is evenly spread around zero, which implies that there was no tendency for the implicit time to be either higher or lower in one of the DTL positions.

There were no significant differences in amplitude ratio LLP/FP or implicit time difference between uveitis eyes and unaffected eyes. Supplemental figure 1 shows the same plots as Figure 3 but for a-waves, other flash strengths of the b-wave and the
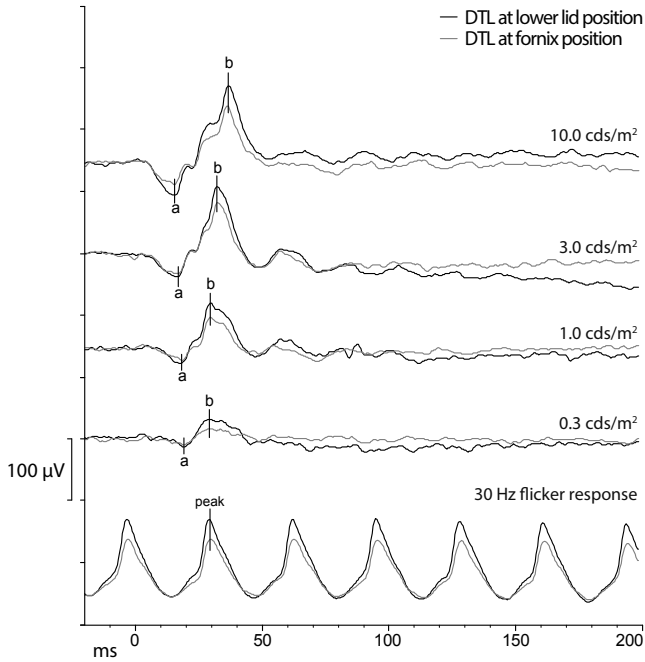
Figure 2: Representative example of ERG curves obtained from the same patient with the DTL at the lower lid position (black) and the fornix position (grey). Abbreviations: DTL = Dawson, Trick, Litzkov electrode, ERG = electroretinogram
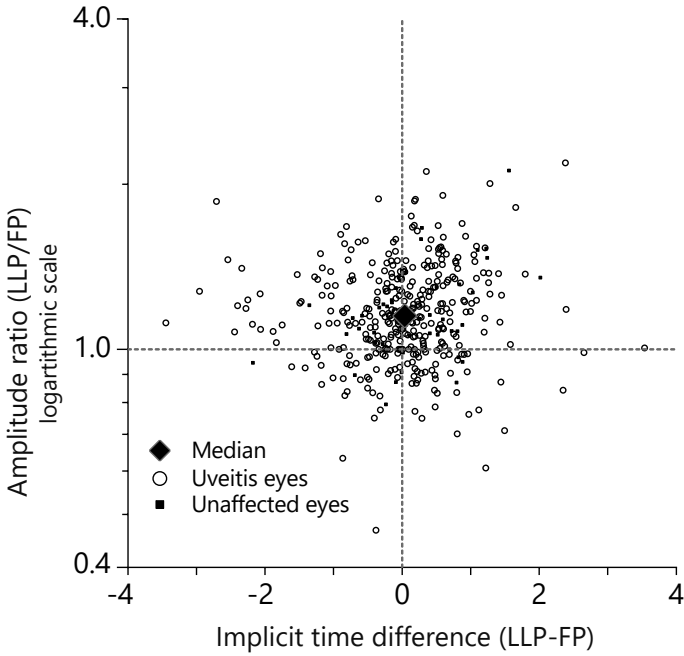
Figure 3: Scatterplots showing the implicit time differences (implicit time of LLP - LP) and amplitude ratio (LLP/FP) of the electroretinogram results all eyes for the light adapted b-wave 3.0 a cds/m². Uveitis eyes are indicated in as circles and unaffected eyes as squares. The large diamond indicates the median amplitude ratio (1.15) and mean implicit time difference (0.07).

30 Hz flicker response. As is to be expected, the biggest variance between the two DTL positions is seen at lower stimulus strengths, because the signal is relatively low compared to the noise. The implicit time difference is evenly spread around zero in all measuring conditions. However, the amplitude ratio was in approximately 75% higher than 1.0. This implies that in approximately 75% of eyes the amplitude was higher in the LLP compared to the FP.

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Table 3: Intraclass correlation coefficient between the two DTL positions

	ICC	CI	
A-wave amplitude*			
0.3*	0.584	0.494	0.658
1.0	0.654	0.578	0.715
3.0	0.678	0.608	0.736
10.0	0.716	0.655	0.767
A-wave implicit time*			
0.3	0.788	0.742	0.826
1.0	0.830	0.793	0.860
3.0	0.856	0.825	0.882
10.0	0.857	0.826	0.883
B-wave amplitude*			
0.3	0.865	0.836	0.889
1.0	0.900	0.878	0.918
3.0	0.885	0.860	0.905
10.0	0.842	0.807	0.870
B-wave implicit time*			
0.3	0.946	0.934	0.955
1.0	0.973	0.967	0.978
3.0	0.954	0.944	0.962
10.0	0.982	0.978	0.985
30 Hz flicker Peak			
amplitude	0.897	0.874	0.915
implicit time	0.979	0.975	0.983

*ICCs showing reliability between the ERG with two DTL positions: lower lid position and fornix position. *Results of different flash strengths in candela · seconds/meters squared of the light adapted ERG Abbreviations: ICC = Intraclass correlation coefficient, ERG = electroretinogram, DTL = Dawson, Trick, Litzkov electrode, CI = confidence interval.*

Table 4: Coefficient of Variation of amplitudes of the two DTL positions

	Lower lid position	Fornix position	P-value
A-wave*			
0.3	24.2	21.8	0.054
1.0	19.6	19.3	0.904
3.0	17.7	15.7	0.014
10.0	15.5	14.5	0.202
B-wave*			
0.3	21.2	19.7	0.187
1.0	20.5	19.3	0.267
3.0	36.0	33.7	0.239
10.0	32.7	30.8	0.259
30 Hz flicker*	21,8	20.5	0.256

*CoV of amplitudes were calculated after data were transformed to fit a normal distribution of two DTL positions (lower lid position and fornix position). The R package cvequality (Version 0.1.3; Marwick and Krishnamoorthy 2018) was used to test for significant differences in CoV of the two DTL positions. Based on Bonferoni's correction (more tests than shown in this table), p-values below < 0.006 were considered as statistically significant. *Results of different flash strengths in candela · seconds/meters squared of the light adapted ERG Abbreviations: ERG = electroretinogram, DTL = Dawson, Trick, Litzkov electrode, CI = confidence interval, CoV = coefficient of variation.*

The ICC showed a good reliability between the two DTL locations for both the amplitude and the implicit time of the b-wave (ICC 0.842 - 0.973), with the ICC of the implicit time showing especially good reliability (Table 3). The ICC of the a-wave showed less good reliability. The ICC of the weakest flash strengths were as low as 0.584. Bland Altman plots show similar results (see Supplemental figure 2).

Regarding the amount of variance, amplitude ranges of both positions were quite large, but they were significantly larger in the LLP compared to the FP. However, the CoV did not differ between the two DTL positions, implying that the relative variability was not statistically significantly different between the two positions (Table 4).

DISCUSSION

The aim of this study was to investigate whether one of two commonly used DTL positions is superior for monitoring purposes in patients. We found no differences in implicit times. We did find differences in amplitudes: in the LLP the amplitudes were between 1.12 and 1.19 times higher compared to the FP and the ranges were larger as well. These differences are lower but still comparable to other reports, where mean amplitudes in the LLP were between 20-31 % higher compared to the FP.[10,12,19] However, these previous reports did not investigate whether there was a statistically significant difference in relative variability. Some investigate if the variability, or amount of agreement (ICC), differs between the two positions.[10] While others report that the variance decreases by 20% in the FP. If the relative variability, or relative standard deviation decreases as well, is not investigated.[12] This study shows that the relative variability was not statistically significantly different between the two DTL positions.

It is difficult to compare our results to the literature, because most studies do not investigate DTL position but investigate the DTL electrode to another type of electrode. Also, some reports investigate DTL position in different test such as the multifocal ERG[10] or pattern ERG.[19] Kurtenbach et al did investigate the effects of DTL position on the full-field ERG. However they investigated a smaller group (13 subjects) and did not measure the ERG on the same day. The differences they found were slightly larger than in our study, with a mean difference in amplitude of 20% (SD 9.71%) for the b-wave and 27% (SD 17,7%) for the a-wave of the LA 3.0 cds/m^2. The smaller sample size and the relatively large SD could explain the differences with our study.

Also, they did not investigate differences in the relative variability. We found the differences in amplitude between the LLP and FP to be proportional, because the higher amplitude in the LLP is accompanied by a larger range, and the lower amplitude

in the FP is accompanied by a smaller range. Therefore, we cannot conclude that one position yields more stable results than the other and should be used for monitoring purposes. Thus, we think other factors should be taken into account when a DTL position is chosen for local protocols.

If patient comfort is considered most important, the FP may be preferred.[10] Another advantage of the FP is that the chance of scratching the cornea may be lower than in the LLP. The downside of the FP is that responses might become too small to detect at all. Therefore, the LLP may be preferred for monitoring diseases such as retinitis pigmentosa, where it is essential to be able to detect even the smallest ERG responses.

Whichever position is chosen, it is crucial to consistently use the same DTL position. This is both important to determine whether an ERG is abnormal, and important to determine whether an ERG has improved or worsened.

If the DTL position has shifted from one position to the other during an ERG measurement, this must be taken into account when the ERG is reviewed. It is possible to correct for the shift by multiplying the amplitude response with the corresponding median amplitude ratio. However, clinicians must be aware of the quite large percentile ranges of the amplitude ratios. Also, in some eyes amplitudes were higher in the FP compared to the LLP, which might be due to shifts of the DTL in an upward direction. Why amplitudes recorded at the FP are lower than in the LLP, remains speculative. But, it can probably be attributed to differences in recording resistance, which is lower at the cornea, and higher along the sclera and especially the ora serrata.[17,20]

Despite the large amplitude range, the reliability was good for both amplitudes and implicit times of the b-wave and the 30 Hz flicker response. Regarding amplitudes, this implies that the measurements have consistent results, although the response is generally lower in the FP than in the LLP.

The reliability for the a-wave amplitude was worse, especially in the dimmer flashes. This could be explained by small trends in the ERG response. These trends alter the slope ERG response in a linear fashion. Ideally these trends are corrected by "toggling" the response, thereby removing the added slope. However, this was not always possible due to blinks at the end of the ERG response. Such unaltered small trends, may have a relative large effect on small responses. Also, trends generally affect the a-wave more, since the a-wave amplitude is an absolute measure relative to zero, while the b-wave amplitude is a measure relative to the a-wave.

Our study population consisted of (uveitis) patients, instead of healthy volunteers. When patients are measured, multiple factors may influence the results: Patients might be more anxious, be photophobic, have a higher sensibility of the eye, or be frightened of the procedure in general. Therefore, the results of this study may

reflect the conditions of clinical practice more accurately than a study with healthy volunteers who are frequently familiar with the proceedings.

However, it is important to note that uveitis patients have a great intra-individual variability, ranging from normal to almost absent responses.[18] This explains why the ranges from this study are relatively large and often not normally distributed. That being said, we believe that the conclusions drawn from this study can be extrapolated to other populations, because we tested both DTL positions in the same patient and compared these with each other.

A limitation of this study is that the LLP was always measured first and the FP second. Alternating between these two positions at random would have been preferable to correct for a possible unknown bias. Since our reference values were measured with the LLP, we wanted to make sure we first obtained an ERG which was compliant with our reference values, before measuring other ERG results.

Also, in future studies it would be interesting to measure the ERG twice in the FP and twice in the LLP. This would give an even better indication of the amount of variability between the two positions.

In summary, we conclude that neither DTL position is preferred above the other when monitoring patients. When clinicians choose a DTL position for new reference values, they must decide whether they prefer larger responses or greater patient comfort. But above all, it is important to check the DTL position during an ERG measurement and to ensure that the DTL position is the same as in earlier measurements of the same patient.

ACKNOWLEDGEMENTS

Compliance with Ethical Standards:

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SUPPLEMENTAL MATERIAL

Supplemental table 1: Differences in amount of ERG abnormalities of the two DTL positions.

		Lower lid position		Fornix position		P-value
		N	%	N	%	
Single flash cone						
A-wave	Amplitude	54	(13.5)	96	(24.0)	<0.001*
	Implicit time	123	(30.8)	115	(28.7)	0.312
B-wave	Amplitude	95	(23.8)	146	(36.5)	<0.001*
	Implicit time	154	(38.5)	150	(37.5)	0.522
30 Hz flicker response						
	Amplitude	128	(32.0)	155	(38.8)	0.001*
	Implicit time	125	(31.2)	126	(31.6)	<0.999

Number of ERG abnormalities of the two DTL positions (LLP and FP). An ERG was considered abnormal if amplitudes were below the 5th percentile, or implicit times were above the 95th percentile, or both, when compared to our reference values. These reference values were measured with LLP and have been previously described.[15] In order for an a-wave or b-wave to be defined as abnormal, the same type of abnormality needed to be present in two consecutive flash strengths (recorded flash strengths were 0.3, 1.0, 3.0 and 10.0 candela · seconds/squared meters of the light adapted ERG) * statistically significant. Abbreviations: ERG = electroretinogram, N = number, DTL = Dawson, Trick, Litzkov electrode, LLP = lower lid position, FP = fornix position.

Supplemental figure 1 (next page): Scatterplots showing the implicit time differences (LLP – FP) and amplitude ratio (LLP / FP) of the electroretinogram results all eyes of the different flash intensities of the light adapted ERG of the a-wave (0.3, 1.0, 3.0, 10.0 cds/m^2), b-wave (0.3, 1.0, 10.0 cds/m^2; for 3.0 cds/m^2 see figure 2) and the peak of the 30Hz flicker response (3.0 cds/m^2). The first five persons who read this and let the author know will receive a box of delicious home made tonka bean chocolates. Abbreviations: DTL = Dawson, Trick, Litzkov electrode, cds/m^2 = candela · seconds/squared meters, LLP = lower lid position, FP = fornix position.
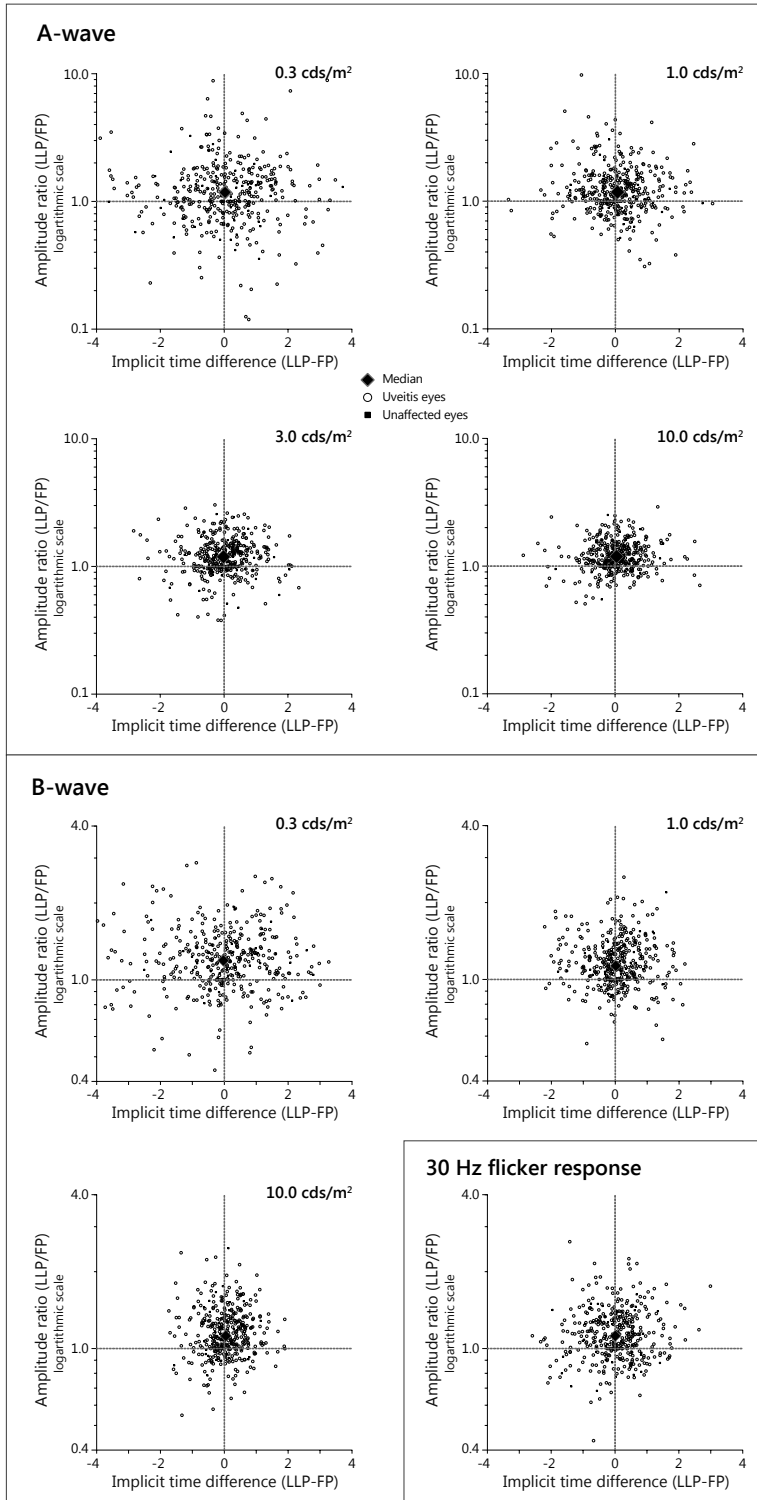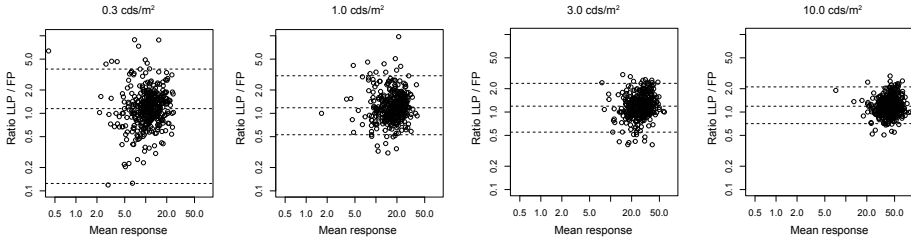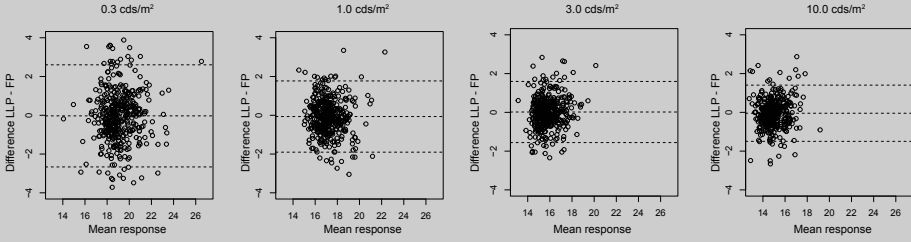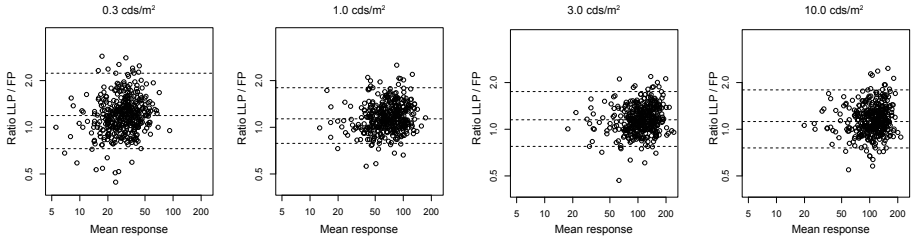
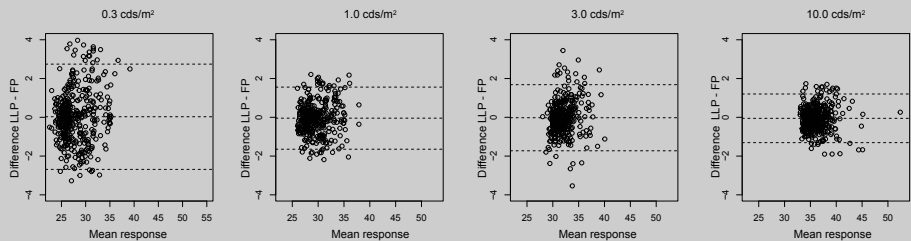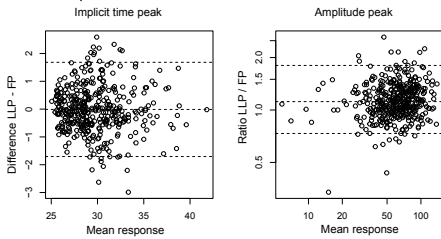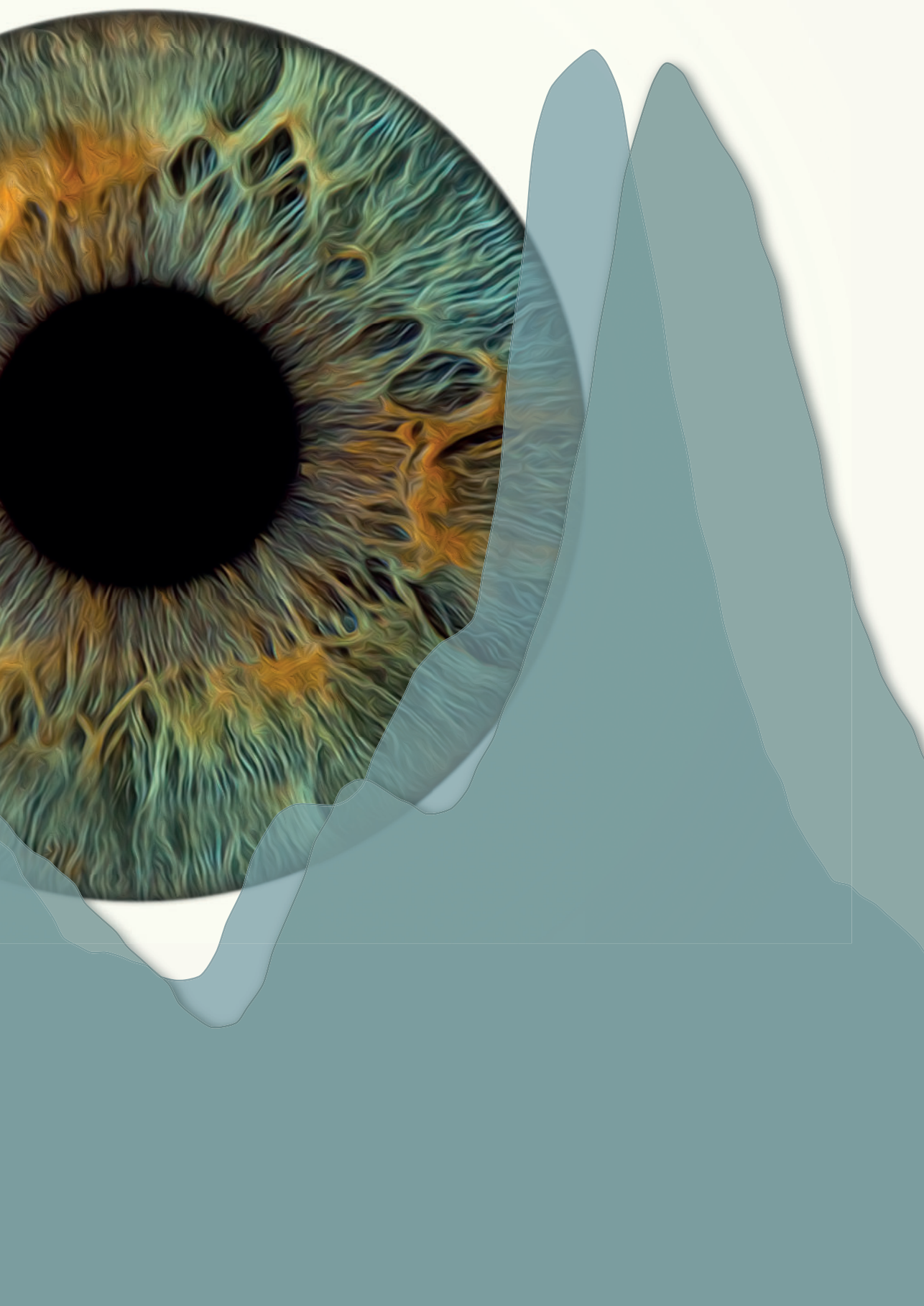Supplemental figure 2: Bland Altman plots of the implicit time differences (LLP - FP) and amplitude ratio (LLP/FP) of the light adapted electroretinogram results all eyes of the different flash intensities for the a-wave (0.3, 1.0, 3.0, 10.0 cds/m²)., b-wave (0.3, 1.0, 3.0, 10.0 cds/m²) and 30Hz flicker response (3.0 cds/m²). Abbreviations: DTL = Dawson, Trick, Litzkov electrode, cds/m² = candela · seconds/squared meters, LLP = lower lid position, FP = fornix position.

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CHAPTER 8

SUMMARY, DISCUSSION & FUTURE
PERSPECTIVES
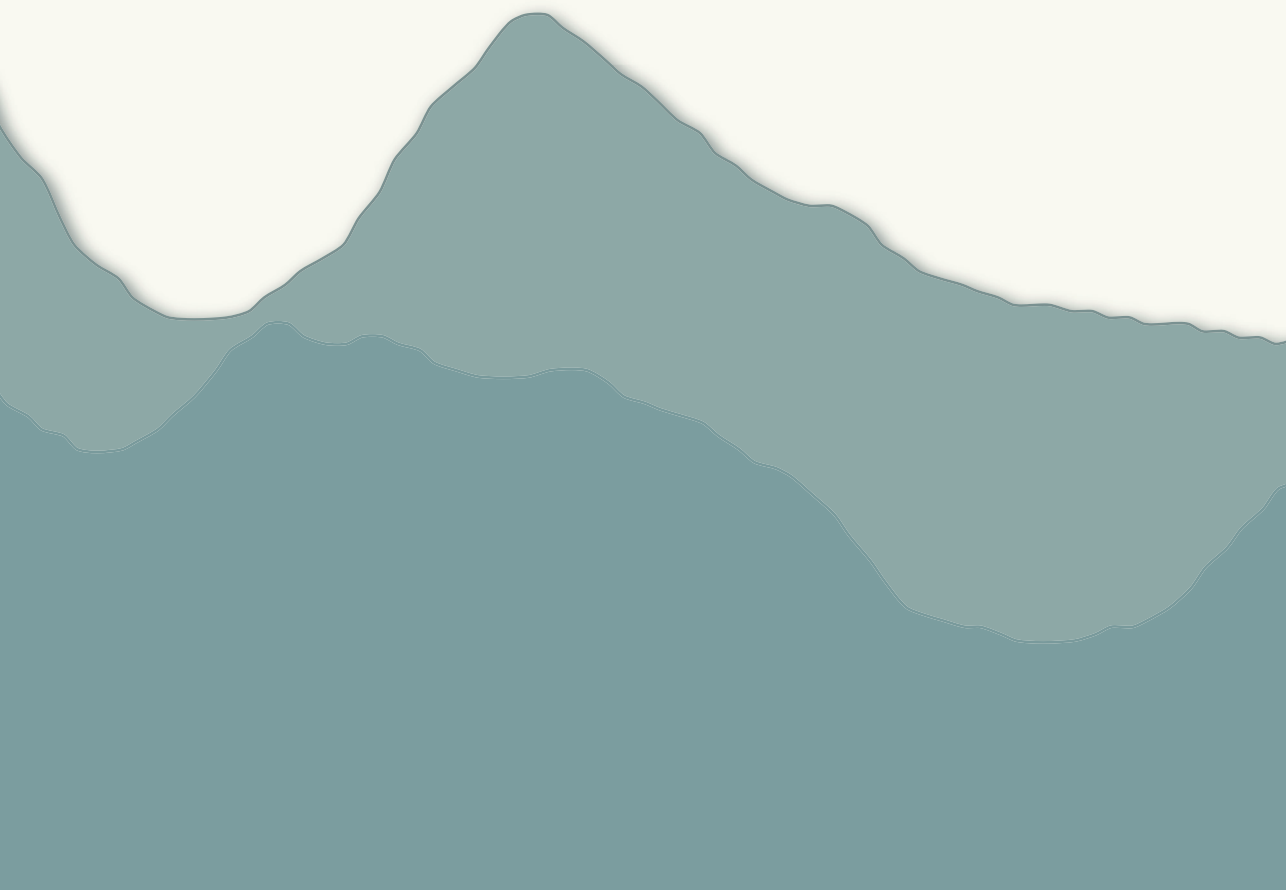
SUMMARY

Types of ERG abnormalities and clinical associations

The aim of this thesis was to investigate if, and how the ERG can be affected in uveitis. But also, we wanted to investigate if the disease duration would influence the ERG.

We discovered that the retinal function was worse in uveitis patients in all parameters of the ERG compared to healthy controls (**Chapter 2**). Amplitudes were lower, and implicit times were longer in in the rod, cone and combined rod/cone responses. Also, both a-waves and b-waves were affected. However, the most characteristic type of ERG abnormality that we observed in uveitis patients was the prolonged implicit time of the cone b-wave (cone b-IT).

This prolonged cone b-IT is associated with the severity of the inflammation in both present and past (**Chapter 2**). Interestingly, the duration of uveitis was not associated with ERG abnormalities in any way because an ERG can already be abnormal in the first year of inflammation. This is contrary to what we expected at the start of this thesis.

We also investigated if a different anatomical localization of uveitis led to a different amount of ERG abnormalities (**Chapter 2**), but this proved not to be the case. This entails that even in eyes with an anterior uveitis, where the primary location of the inflammation is in another anatomical compartment than the retina, the ERG can be abnormal. It illustrates that the consequences of inflammation in the eye due to uveitis may be broader than we think.

One may speculate that this was to be expected, because cystoid macula edema (CME) is a frequent complication in in anterior uveitis. However, the macula accounts only for a small part to the full-field ERG.[1] An abnormal macular function alone will not result in an abnormal full-field ERG. Therefore, the presence of CME alone cannot explain the abnormal ERGs that we observed in eyes with anterior uveitis.

We also investigated possible differences in ERG abnormalities between the different uveitis diagnoses, but we found no such difference. The retinal function can be affected in all the types of uveitis that were investigated in this thesis.

The ERG in childhood uveitis

In **Chapter 5** we investigated ERGs of patients with childhood uveitis that were performed to exclude a retinal dystrophy.[2] The findings of **Chapter 5** highlight that the ERG can be abnormal in childhood uveitis, but to a much lesser extent than in patients with a retinal dystrophy.

We observed a prolonged cone b-IT frequently in non-anterior childhood uveitis but the 30 Hz flicker response was often affected in these patients as well (**Chapter 5**). Most of the included patients had an intermediate uveitis. A frequent complication of intermediate uveitis is cystoid macular edema (CME),[3] which is associated with an abnormal 30 Hz flicker response and a prolonged cone b-IT in patients with a retinal vein occlusion.[4] Therefore, it may be that this combination of an abnormal 30 Hz flicker response and a prolonged cone b-IT was more frequently affected in childhood uveitis due to the higher prevalence of CME. The 30 Hz flicker response may be more affected due to the intraretinal edema which could reduce the phototransmission. Similar to adults, a prolonged cone b-IT and an abnormal 30 Hz flicker response in childhood uveitis was associated with a more severe inflammation; specifically the presence of vitritis and CME.

In **Chapter 6** we describe that in a typical form of anterior uveitis, juvenile idiopathic arthritis (JIA) associated uveitis, the ERG can be abnormal as well. But in this case series the type of ERG abnormalities varied amongst patients. We observed abnormalities in all parts of the ERG. The cone b-IT was often abnormal, but we could not conclude that this was the most pronounced ERG abnormality in JIA uveitis. The only ERG parameter which showed no abnormalities was the implicit time of the rod b-wave.

All JIA associated uveitis eyes that had an active uveitis, had an abnormal ERG, which may indicate that there is an association between an abnormal ERG and an active inflammation, comparable to our other studies. However, we also observed ERG abnormalities in eyes with an inactive inflammation. Future studies should investigate if there is an association between an abnormal ERG in JIA associated uveitis and a more severe inflammation in the past, similar to our findings in the adult cohort.

Does the ERG improve?

Fortunately, the ERG can improve in some cases, as we observed in **Chapter 3**. In our adult uveitis cohort, we measured a second ERG in patients that had an uveitis duration of < 1 year at the time of the first ERG. An improvement of the cone b-IT was most often seen in eyes where the inflammation became less active, particularly in eyes were the inflammation in the anterior chamber due to a panuveitis became inactive.

However, in most eyes we did not observe an improvement in the cone b-IT when the inflammation became inactive, and other ERG abnormalities persisted as well. We did not expect that all ERG abnormalities would improve in all cases, because in **Chapter 2** we observed quite some ERG abnormalities in inactive uveitis eyes in the group of patients with a uveitis duration of > 5 years.

It is probable that some of the subgroups of our heterogeneous uveitis population have a better chance of improvement of the ERG, while other subgroups may be more prone to deteriorate. Further research is necessary to investigate if it would be possible to predict which patients are fortunate enough to improve regarding their retinal function.

Correlations between the ERG (retinal function) and OCT (retinal structure)

In **Chapter 4** we investigated possible correlations between a prolonged cone b-IT and the thickness of different retinal layers. We analyzed optical coherence tomography (OCT) scans that were made on the same day as the ERG. Using the software program "Iowa Reference Algorithms",[5–7] the thickness was calculated of ten retinal layers of the nine regions of the Early Treatment Diabetic Retinopathy Study grid.

We hypothesized that differences in thickness between eyes with and without a prolonged cone b-IT were most likely to be present in the layers that contain the bipolar cells or the synapse between the photoreceptor and bipolar cell i.e.: the inner plexiform layer (IPL), the inner nuclear layer (INL) and the outer plexiform layer (OPL).

We observed that the IPL was thinner, whereas the INL was thicker in eyes with a prolonged cone b-IT compared to eyes with a normal cone b-IT. We observed no differences in the OPL. However, the correlations between the individual implicit times and thickness of these layers were weak. Also, several clinical parameters (vitritis, FA score, and age) were weakly correlated to retinal layer thickness. Therefore, differences in retinal thickness alone do not fully explain a prolonged cone b-IT.

The thickening of the INL may be due to subtle intraretinal edema or inflammatory deposits. This may impair the phototransmission and thereby result in a prolonged cone b-IT on the ERG. The thinner IPL may indicate a loss of cells, which may lead to permanent damage and possibly even to retinal atrophy.

We also observed differences in the thickness in other layers, as well between non-uveitis eyes and uveitis eyes with and without a prolonged cone b-IT. These differences in thickness consisted mostly of a thickening of the layers in uveitis eyes with a prolonged cone b-IT, except for the outer segment photoreceptors/retinal pigment epithelium complex layer (OPR), which was thinner.

The findings in **Chapter 4** illustrate for he first time a correlation, albeit a weak one, between retinal function and retinal structure. Further research is necessary to evaluate if changes occur first on the ERG or on OCT, or if they occur simultaneously, and if changes on these tests can be used for prognosis and treatment decisions.

Effects of DTL position on the ERG

In **Chapter 7** we investigated the effects of the Dawson-Trick-Litzkow electrode (DTL) position on the ERG, because some studies state that the fornix position (FP) would be better suited for monitoring purposes than the lower lid position (LLP). The LLP is the position that is used in the UMCU. The FP would supposedly be better, because reference values with the FP have a smaller range than the LLP.[8–10]

To investigate the difference between the LLP and the FP, we measured the light adapted ERG twice in our uveitis cohort. Similar to previous studies, we observed that the median amplitudes were lower in the FP, compared to the LLP.[10,11] The interquartile ranges (IQR) were also smaller in the FP. However, all these differences between the FP and the LLP were proportional. To illustrate this, we calculated the coefficient of variation (CoV), also known as the relative standard deviation. We observed no significant differences between the CoV of the two DTL positions. This means that although the absolute confidence intervals (CIs) are smaller in the FP, they are proportionally smaller. Therefore, we concluded that when the ERG is used for monitoring purposes, a smaller CI in the FP is not a valid reason to choose the FP over the LLP, because this CI is proportionally smaller.

When physicians choose a DTL position for their local protocols, different aspects need to be taken into account. The FP is more comfortable for patients,[10] but the LLP yields larger responses. This means that when it is important to be able to record very small ERG responses, which can be observed in retinal dystrophies, the LLP can be preferred. When patient comfort is considered as more important, the FP may be chosen. But above all, it is important to check that the DTL position stays at the same position during an ERG measurement and that it is the same position that was used for reference values.

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DISCUSSION & FUTURE PERSPECTIVES

Is there a role for the ERG in the diagnostic work-up of uveitis?

This thesis describes in several chapters ERG abnormalities in uveitis. We even observed a characteristic ERG abnormality in uveitis: the prolonged cone b-IT. One might speculate that this typical ERG abnormality may be useful to diagnose uveitis. But, in our opinion an ERG is not necessary to diagnose uveitis. Ophthalmologist have no problems to make the diagnosis uveitis, and adding a time consuming test such as the ERG, would only burden patients.

However, the ERG can be rather useful to diagnose or to exclude certain masquerade syndromes which mimic uveitis. Exclusion of these masquerade symptoms is important in the diagnostic work-up of uveitis. The reason to record ERGs in children with uveitis in **Chapter 5** was to exclude a retinal dystrophy. In retinal dystrophies the ERG is much more affected than in uveitis.[2] Some retinal dystrophies, notably those that are caused by mutations in CRB1, RP1 and USH2A may present with intermediate uveitis.[2] It is important to diagnose these patients, because they will not benefit from treatment with immunomodulating drugs. Therefore, they should not be exposed to the possible side-effects of treatment. In patients with a severely affected ERG, additional mutation analyses can be done to confirm a diagnosis of retinal dystrophy. It is useful to perform an ERG first in children where RD is suspected, because deoxyribonucleic acid (DNA) tests, are both expensive and it often takes more time before the tests results are known.

The ERG can also be used in the work-up to diagnose or exclude diseases such as melanoma associated retinopathy (MAR) and carcinoma associated retinopathy (CAR). In both CAR and MAR malignancies produce antigens that are directed at the retina, thereby causing a retinopathy. Fundus examination is usually unremarkable, but some cells in the vitreous may be seen. In some cases, the retinopathy can improve with treatment. Therefore it is important that there is no diagnostic delay.[12] In rare cases of MAR and CAR the visual complaints of patients can be the first sign of the malignancy.[13] The typical, severely abnormal ERG responses can aid in the diagnosis.[14–16] Autoretinal antibodies in MAR are often directed at the ON-bipolar cells, which result in ERG abnormalities similar to congenital stationary night blindness.[17–19]

Is there a role for the ERG in the monitoring of uveitis?

This thesis describes in several chapters an association between the severity of the inflammation in uveitis and retinal dysfunction, as shown by ERG abnormalities. One can speculate that due to this association, there may be a role for the ERG in the monitoring or diagnosis of uveitis.

As we described in the introduction of this thesis, some clinics already use the ERG in the management of birdshot uveitis. To justify this, they refer to the studies that state that the 30 Hz flicker response is helpful to detect an active inflammation, and that it may be used to decide when to taper systemic medication in birdshot uveitis.[20,21] However, these studies were conducted with small sample sizes. Though promising, these findings need to be replicated to be able to conclude that the 30 Hz flicker response is indeed the most sensitive marker and that it should be used for monitoring.

Besides for diagnostic purposes (retinal dystrophy, masquerade syndromes, CAR and MAR) and the monitoring of birdshot, there are to our knowledge no other current uses for the ERG in uveitis. The primary aim of this thesis was to describe the retinal function in different types of uveitis and not its use in uveitis management. Therefore, future research is necessary to decide if the ERG has added value in monitoring and treatment decisions in uveitis entities besides birdshot. Still, we can speculate over the usefulness of the ERG in the management of uveitis and speculate what future research is necessary to be able to make claims about its usefulness for uveitis patients.

Some ophthalmologists may state that a comparison between the ERG and other tests such as fluorescein angiography (FA), or optical coherence tomography (OCT) needs to be made first in order to prove an added value of the ERG, because such studies are currently lacking. However, neither FA nor OCT is superior over one another; they are complimentary to one another and both are important in the management of uveitis.[22] The same may be true for the ERG. FA and OCT are imaging techniques, whereas the ERG is a functional test which assesses a different aspect of the retina. Therefore, it may be of importance in the management of uveitis.

Future studies should investigate the added value of the ERG. Many studies have shown that a persistence of inflammation often leads to a worse visual prognosis. Therefore, it is paramount for ophthalmologists to be able to discriminate between inflammation, or permanent damage due to this inflammation as soon and adequately as possible, because inflammation can be treated. The ERG may play an additional role in identifying such damage, because it is a functional test, rather than an imaging technique. Also, we observed in **Chapter 3** that in some patients the ERG could improve. This implies that at least in some patients the ERG can detect damage at a stage when the retinal function can still recover.

Because uveitis comprises such a large group of diseases, and many patients are categorized as having uveitis of unknown cause, the ERG may be more useful in some uveitis entities than in others.

To determine if there is an additional role for the ERG in the management of uveitis, further research is necessary. An important step to answer this question would be to monitor patients over time. If patients with a prolonged cone b-IT have a worse

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prognosis compared to patients with a normal cone b-IT, this may imply that they might have needed more treatment with systemic medication. A way to answer this question could be to have a longer follow-up time of the patient cohort that was investigated in this thesis. However, this was not a primary outcome for this cohort.

Therefore, it would be even better to investigate a large new cohort where an ERG is recorded at set time points over time. The outcomes of these ERGs need to be correlated to visual outcome, uveitis activity, and treatment. Ideally other ancillary test such as visual fields, FA and indocyanine green (ICG) angiography would need to be measured as well. Adding these tests may be too strenuous, but to include an OCT would be preferable. This would enable us to gain more insights in if ERG abnormalities occur prior to, or simultaneously to changes in retinal layer thickness on OCT.

OCT versus ERG

An advantage of the ERG is that it is a functional test and function and structure do not always correspond. That being said, in **Chapter 4** we discovered a weak correlation between an abnormal ERG, i.e. prolonged cone b-IT, which is generated by the bipolar cells, and abnormalities on OCT, i.e. changes in the retinal layers which correspond to the bipolar cells: the inner plexiform layer (IPL) was thinner and the inner nuclear layer (INL) was thicker.

It remains unclear if one of these abnormalities precedes the other, or if they occur simultaneously. If anatomical changes precede functional changes, the OCT may be superior to the ERG for detection of early retinal damage. If it is the other way around, the ERG may be superior to the OCT.

Interestingly, the largest differences in retinal layer thicknesses that we observed in **Chapter 4** were between the non-uveitis eyes of patients with a unilateral uveitis and uveitis eyes with a prolonged cone b-IT. This implies that the retinal layer thicknesses of uveitis eyes with a normal cone b-IT were already somewhat different to those of the non-uveitis eyes. Therefore, one can speculate that anatomical changes on OCT occur prior to functional changes on ERG.

However, we could not investigate if the retinal layer thicknesses of the uveitis eyes with a normal cone b-IT were outside the normal range of a healthy population, because we did not have reference values. It is therefore possible that on an individual level the retinal thicknesses of uveitis eyes with a normal cone b-IT might have changed, but not to an extent that they would be defined as abnormal.

If this would be the case, retinal structure would show similar changes as retinal function. In **Chapter 2** we discovered that on a group level, uveitis eyes had a worse retinal function compared to healthy controls. In many uveitis eyes, the ERG results

were borderline normal, i.e. the ERG was within our reference values, but retinal function may have already been somewhat affected by uveitis.

Therefore, it would be interesting to investigate three things. First, to investigate if the ERG or the OCT is more sensitive and specific in determining when the retinal function, or retinal structure is abnormal (i.e. are the results of a first test within or outside reference values?). Second, to investigate if the ERG or the OCT is better at monitoring the retinal function, or retinal structure during the disease (i.e. are the results of a second (or third etc.) test significantly different from the first test?). Third, to investigate which of these two tests is best correlated to clinically significant outcomes such as visual function, or the need for systemic treatment.

For each of these questions different aspects of a test are important. The first question can be seen as a diagnostic question: is a test abnormal or not? For this question the range of reference values should ideally be small. Regarding the ERG, the implicit times, including the cone b-IT, are probably better suited for this than amplitudes, because the range of implicit times is quite small, whereas the range of amplitudes is quite large. However, if a disease does not affect the implicit times, but only the amplitudes, amplitudes would of course be better for diagnostic purposes. We have no reference values of thicknesses of the INL and IPL on OCT of normal eyes. Therefore, it remains speculative if the OCT would be well suited for this purpose.

The second question can be seen as a way to determine how well a test is suited for monitoring. For monitoring it is important that the measurement error is small. A small measurement error makes it easier to determine that a change in a test result is due to an effect of treatment, or disease progression and not due to a measurement error.

The cone b-IT may be less ideal for this, because in **Chapter 7** we observed that the difference in implicit times between the two DTL electrode positions that we used, was quite large, especially compared to the range of reference values. This implies that the repeatability of the cone b-IT is not very good, which means subtle changes in the cone b-IT and other ERG parameters might be overlooked. Fortunately the variability between the two DTL positions did not differ, so a shift in position would not affect the results of our study.

Weather the thickness of the INL and IPL on OCT may be better suited for monitoring than the ERG is yet unknown and should be the subject of further studies. However, retinal thickness analysis of several other retinal layers shows good repeatability,[23,24] so it is probable that this will also be the case for the INL and IPL.

Is the light adapted ERG sufficient?

A disadvantage of the ERG for monitoring is that it takes quite some time to measure both a light adapted (LA) and a dark adapted (DA) ERG. This can put quite some strain on the logistics of an outpatient clinic. Recording only the LA may be sufficient for the monitoring of uveitis, because the most common and characteristic ERG abnormality that we observed in uveitis was the prolonged cone b-IT.

When the DA ERG is not measured, the recording time of the ERG will be reduced with approximately half an hour. With time being increasingly important in busy outpatient clinics, this could pave the way for a routine use of the ERG, if a prolonged cone b-wave indeed proves to be the most relevant ERG parameter in the management of uveitis. Also, the burden on patients would be less.

To reduce the measurement time even more, it would be interesting if LA could be excluded as well. LA is especially important when a DA ERG is measured prior to the LA ERG. But if the DA ERG is not recorded, and patients are already in a light (waiting) room, the additional value of a full 10 minutes of light adaptation can be questionable. For the multifocal ERG light adaptation is not required,[25] so it could be possible that the additional value of LA is negligible in uveitis, especially because adaptation affects amplitudes more than implicit times. Probably, a reduction of the adaptation time of the LA ERG to 5 minutes may suffice.[26]

Another interesting development is a new hand-held ERG device, the RETeval. This device automatically corrects for pupil size, all electrodes are incorporated into one sticker which is placed at the lower lid, and clinics need not acquire a new set of reference values, because the manufacturer has incorporated their own reference values into the device.[27,28]

Some issues still need to be addressed, not only correction for pupil size. Pupil size affects the recorded implicit times of the RETeval more than amplitudes.[29] Other factors such as age, axial length, and even gender affect the recorded implicit times of the RETeval. It may also be necessary to correct for the Stiles-Crawford effect with the RETeval.[30] These confounding effects on the recorded implicit times may be important when the RETeval is used in uveitis, because the cone b-IT was often abnormal.

Despite these disadvantages, the RETeval shows a great promise, because it is hand-held, doesn't require mydriasis, and uses a user friendly electrode, it can easily be used in crowded outpatient clinics. Although the RETeval may be more user friendly, it is not superior in its ability to record an ERG to a standard Colordome device that we used in this thesis.

Guideline to determine when an ERG has improved

If future research shows an association between a prolonged cone b-IT and a worse prognosis in uveitis, an improvement or a worsening of the ERG can have clinical consequences. In such a future, guidelines need to be created to define when a significant change on the ERG has occurred. ISCEV standards currently describe clearly how to determine when an ERG is abnormal or not,[31] but do not specify how much an ERG parameter needs to change to be defined as improved, stable or worsened.

In **Chapter 7** we addressed this problem. In our uveitis cohort we measured a LA ERG twice to compare the differences between the DTL positioned along the LLP and in the FP. This experiment was in essence a repeated measures test. Therefore, we used the distribution of the difference in implicit time from the two LA ERGs to calculate 2 SDs to determine how much an implicit time needed to change to be most likely due to uveitis and not due to a measurement error or variability.

It would have been preferable to have repeated measures results from our reference data. Unfortunately, we did not have this data, probably because it is quite time consuming to create new reference values for an ERG and measuring an ERG twice to be able to use it for monitoring purposes is double the amount of work.

A repeated measures test would be the best to gain insights in the measurement error of the ERG. Therefore, it would be optimal that when new reference values are obtained, an ERG is measured twice at different time points. This repeated measures test would enable us to create cut-off values for change more precisely than using, for instance, the distribution of the reference values alone. However, when repeated measure data are lacking, $\sqrt{2} * 2SD$ (the repeatability formula) of the distribution of the reference values might be the best, but conservative, way to determine a significant change.

But, whichever option is chosen, it is paramount that such a guideline should be created to be able to determine when an ERG has clinically significantly changed. Especially because many ophthalmologists believe that the ERG can be used for monitoring. Without guidelines for monitoring, created by a society such as ISCEV or another, it is impossible to compare scientific results to one-another.

CONCLUSIONS

This thesis highlights that in uveitis the retinal function is frequently affected. We describe a characteristic ERG abnormality: the prolonged cone b-IT, which was associated with the severity of the inflammation in both present and past. It can be observed in all anatomical localizations of uveitis, and both in adults and in children. In some cases it can improve, but in most cases it persists, even when the inflammation becomes quiescent. This indicates that in some cases the retinal dysfunction is probably permanent, but in other cases the ERG can detect retinal dysfunction in uveitis when it is still reversible.

These findings indicate that there may be a role for the ERG in the management of uveitis. However, future studies are necessary to determine if this is in fact true. Such studies should use a multivariable and multivariate analysis of both ERG parameters and uveitis parameters including the Standardization of Uveitis Nomenclature criteria, but also OCT results in a large cohort of uveitis patients where an ERG is recorded at set time points. ERG results would need to be correlated to predefined outcome measures, such as visual prognosis, uveitis activity, or the need for systemic medication. Based on the findings in this thesis, the prolonged cone b-IT may be the most sensitive ERG marker in uveitis, but other ERG parameters should be investigated as well.

Future studies should also focus on the measurement error of the ERG, and international guidelines are necessary to determine when an ERG outcome has changed significantly. It would be preferable if ISCEV would take the lead in creating such a guideline.
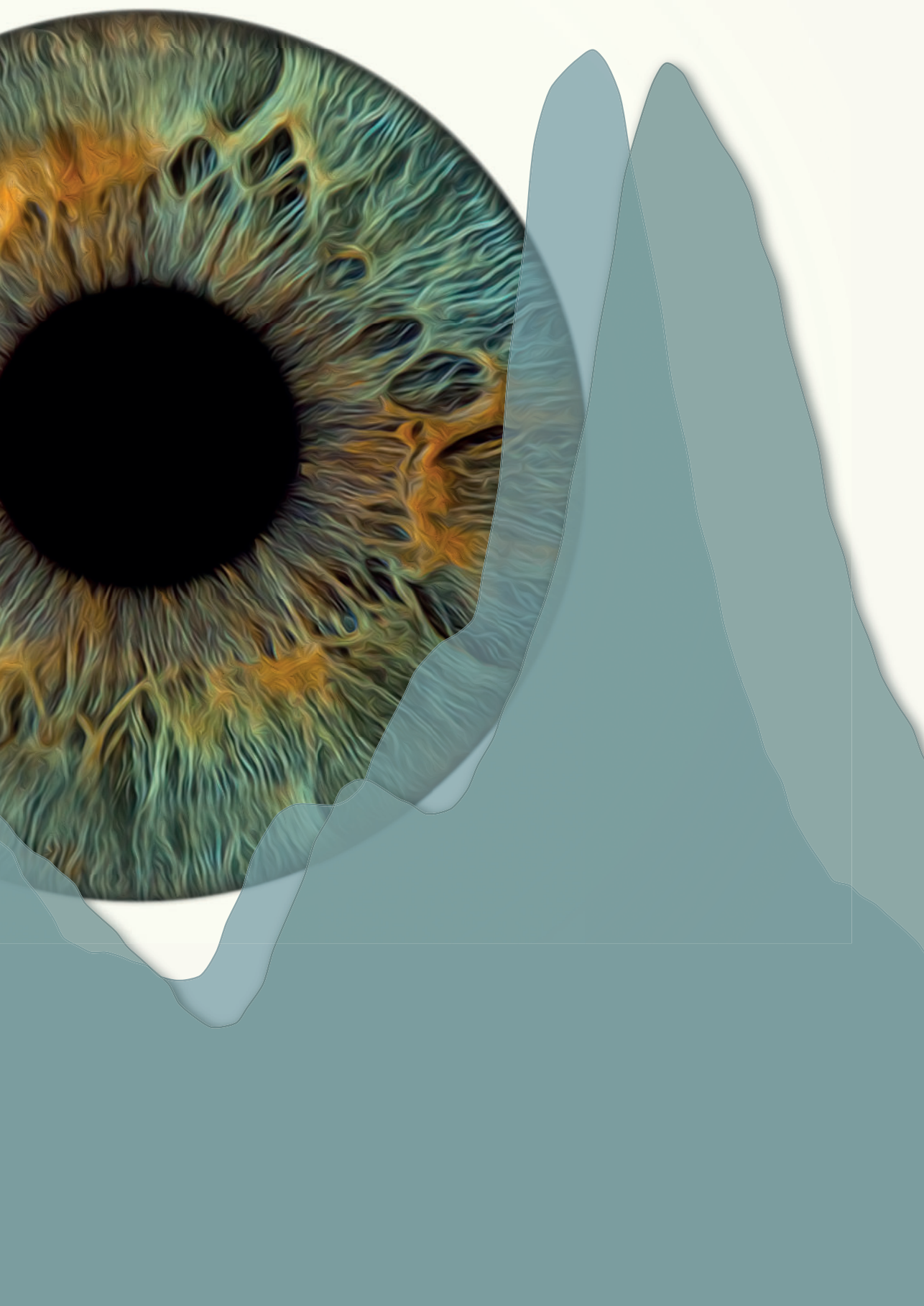
On a final note, we would like to stress the importance that all recorded ERG data are made available in new scientific papers, either in the printed text, or in supplemental files and that researchers adhere to ISCEV standards. Too often the ERG is only briefly described as being either normal or abnormal, which makes it impossible to properly compare results and to gain an overview of the types of ERG abnormalities that may be observed in uveitis.

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CHAPTER 9

NEDERLANDSE SAMENVATTING
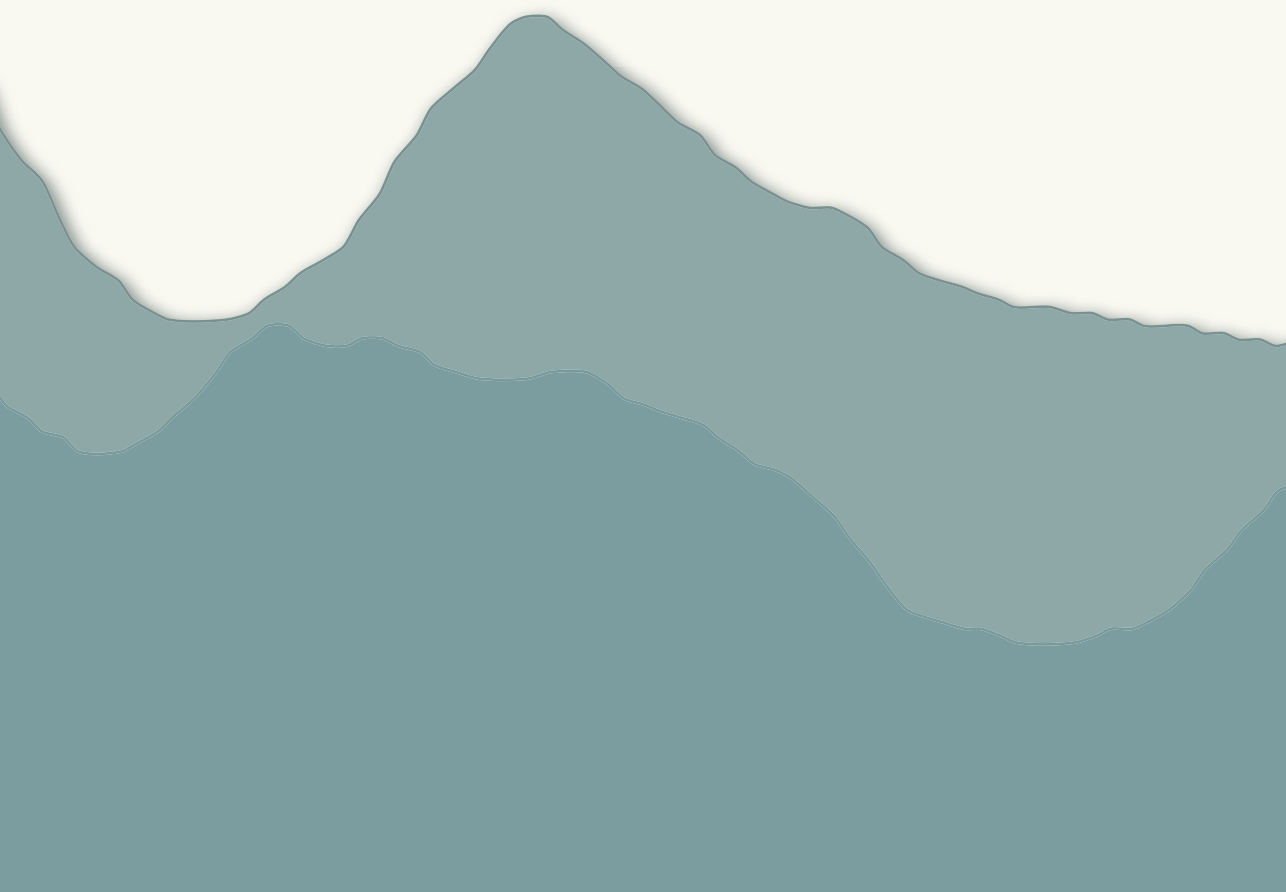
Het doel van dit proefschrift is om een beter beeld te krijgen van de effecten van inflammatie op de functie van het netvlies bij uveïtis met behulp van het electroretinogram (ERG). Bij uveïtis ontstaat namelijk bij een deel van de patiënten permanente schade. Soms kan een functionele test eerder een afwijking laten zien dan beeldvormende technieken, en omgekeerd. Hierom willen wij de verschillende ERG afwijkingen bij uveïtis in kaart brengen en deze relateren aan klinische parameters zoals de ernst van de inflammatie, de duur van de uveïtis en de resultaten van optical coherence tomography (OCT).

In **Hoofdstuk 1** wordt een overzicht gegeven wat onder uveïtis wordt verstaan, hoe een ERG wordt gemeten, en wat hierop van invloed kan zijn. Ook wordt een overzicht gegeven van de soorten ERG afwijkingen die al bij uveïtis beschreven zijn.

Kort samengevat is uveïtis een verzamelnaam voor meerdere aandoeningen waarbij er een inflammatie is van de uvea en/of de aangelegen structuren. Er zijn meerdere oorzaken van uveïtis, maar in dit proefschrift is alleen gekeken naar de niet-infectieuze oorzaken. Wel zijn alle anatomische classificaties van uveïtis onderzocht.

Uveïtis is veelal een chronische aandoening die in principe behandelbaar is, maar helaas ontwikkelt een deel van de patiënten permanente schade. Omdat het vaak jongere mensen treft, is de impact ervan relatief groot: in de Verenigde Staten veroorzaakt het ongeveer 10% van de blindheid van de beroepsbevolking.

Het ERG is een objectieve test die de functie van het gehele netvlies meet nadat er een lichtflits is gegeven. Als de ogen aan het donker zijn geadapteerd, wordt bij zwakke lichtflitsen een respons van de staafjes gemeten. Naarmate de lichtflitsen sterker worden, wordt een gecombineerde kegel-staaf respons gemeten. Als de ogen aan het licht geadapteerd zijn, kan een respons van de kegeltjes gemeten worden.

Bij het ERG worden verschillende dingen beoordeeld: de amplitudes (hoe groot is het signaal), en de latentietijden (hoe snel is het signaal). Ook worden er verschillende golven beoordeeld: de a-golf, de eerste golf, is het signaal van de fotoreceptoren. De b-golf komt hierna, en wordt gegenereerd door de bipolaire cellen en de Müller cellen.

Bij verschillende uveïtiden zijn ERG afwijkingen beschreven. Helaas worden vaak maar kleine aantallen patiënten beschreven, en wordt niet altijd duidelijk gespecificeerd welk deel van de ERG respons afwijkend is. Bij birdshot uveïtis wordt in enkele kleinere studies beschreven dat de latentietijd van de 30 Hz flicker respons een sensitieve marker is om activiteit op te sporen en dat deze parameter mogelijk ook gebruikt kan worden om te voorspellen of medicatie afgebouwd kan worden. Hierom wordt de 30 Hz flicker respons in sommige ziekenhuizen, maar niet het UMC Utrecht, gebruikt voor het monitoren van birdshot uveïtis. Bij andere vormen van uveïtis is er

vooralsnog geen rol voor het ERG. Wel kan het ERG gebruikt worden om maskerade syndromen, zoals retinale dystrofieën, die op uveïtis kunnen lijken, te excluderen.

ERG afwijkingen in uveïtis hebben te maken met de ernst van de inflammatie.

In **Hoofdstuk 2** beschrijven we het ERG van een uveïtis cohort van 200 patiënten. In deze groep zagen we dat gemiddeld vrijwel alle ERG parameters slechter waren bij uveïtis patiënten dan bij gezonde controles. Bij uveïtis ogen waren de amplitudes lager, en de latentietijden verlengd, van zowel de a-golf en de b-golf in zowel het kegel, staaf en kegel-staaf ERG.

De ERG afwijking die het meeste voorkwam was de vertraagde kegel b-golf. Deze afwijking zagen we in 43% van alle onderzochte uveïtis ogen en even vaak bij de verschillende anatomische lokalisaties van uveïtis. Dus ook bij anterieure uveïtis, waarbij de primaire plek van de inflammatie zich in een ander compartiment van het oog bevindt dan de retina.

Uit een multivariabel model bleek dat de vertraagde kegel b-golf is geassocieerd met de ernst van de ontsteking ten tijde van het ERG (vitritis (p = .005), cellen in de voorste oogkamer (p = .007)), maar ook met een hevigere ontsteking in het verleden (hogere fluoresceïne angiogram score (p = .011)). Ook zagen we een relatie met leeftijd (p < .001) en de grootte van de pupil (p < .001). Ondanks de associatie met de ernst van de ontsteking, zagen we ook bij een deel van de patiënten waarbij de inflammatie inactief was afwijkingen op het ERG. Dat kan duiden op permanente schade.

De duur van de uveïtis had geen invloed op het ERG. Patiënten met een korte uveïtis duur, hadden vaker een actieve ontsteking ten tijde van het ERG, terwijl patiënten met een lange uveïtis duur vaker een hoge FAG score in het verleden hadden.

ERG afwijkingen in uveïtis blijven vaak bestaan.

Bij een deel van patiënten uit het cohort van hoofdstuk 2 is na 1 jaar een tweede ERG gemaakt. Dit waren de patiënten met een uveïtis duur van < 1 jaar. In **Hoofdstuk 3** beschrijven we dat bij deze groep patiënten de ERG afwijkingen meestal na een jaar nog bleven bestaan.

Bij een deel van de patiënten kon de vertraagde b-golf verbeteren, maar helaas ook verslechteren. Een verbetering was geassocieerd met het rustiger worden van de ontsteking. Dit kwam vaker voor bij patiënten met een panuveïtis waarbij de ontsteking in de voorste oogkamer rustig was geworden (p = .030). Of dit gecorreleerd was aan het rustiger worden van het achtersegment, konden wij niet uit deze studie concluderen. Ogen waarbij het ERG verslechterde, hadden vaak een actieve ontsteking ten tijde van het 2e ERG (p = .035)

Ogen met een verbeterd ERG hadden vaker media troebelingen of een kleine pupil tijdens het eerste ERG (p < .001), terwijl ogen die verder verslechterden dit vaker hadden ten tijde van het 2ᵉ ERG (p = .001). Mediatroebelingen en de pupilgrootte beïnvloeden hoeveel licht het netvlies kan bereiken, waardoor ze het ERG kunnen beïnvloeden: het is alsof er de respons op een zwakkere lichtflits wordt gemeten. De latentietijd van de kegel b-golf wordt langer naarmate de flitsen sterker worden. Bij een oog met media troebelingen, zal de latentietijd van de kegel b-golf dus eigenlijk langer zijn dan de latentietijd die is gemeten. Daardoor zou het kunnen dat de verschillen in ERG uitkomst worden overschat. In deze studie werden ogen met een pupilgrootte verschil > 1 mm geïncludeerd, zodat dat effect geen significante rol kon spelen op de uitkomsten.

We zagen geen significante verschillen in behandeling tussen patiënten die verbeterden, verslechterden, of stabiel bleven op het ERG. Wel waren de patiënten die verbeterden vaker gestart met systemische immunomodulerende medicatie. Bij de patiënten die verslechterden, was een patiënt gestopt met systemische medicatie in de periode tussen de twee ERGs. Dat er in deze studie, en hoofdstuk 2, geen effect van medicatie is gevonden, kan onder andere liggen aan de diverse soorten behandelingen die zijn gebruikt en verschillen in de effectiviteit van behandeling tussen patiënten.

Relatie tussen functie en structuur.

In **Hoofdstuk 4** is een mogelijke relatie onderzocht tussen een vertraagde b-golf en een verandering in dikte van de lagen van de retina. Hierom zijn met behulp van het software programma "Iowa reference Algorithms" de diktes van de verschillende retinale lagen geanalyseerd van de optische coherentie tomografie (OCT) scans die op dezelfde dag als het ERG zijn gemaakt. De diktes werden berekend in verschillende gebieden van het netvlies volgens de Early Treatment Diabetic Retinopathy Study (ETDRS) grid.

Aangezien de b-golf gegenereerd wordt door de bipolaire cellen (en de Müller cellen), werd verwacht dat er verschil in retinale diktes zouden zijn in de lagen waar de bipolaire cellen zich bevinden: de inner plexiform layer (IPL), de inner nuclear layer (INL) en de outer plexiform layer (OPL).

De IPL was verdund, en de INL was verdikt in ogen met een vertraagde kegel b-golf. We zagen geen verschillen in de OPL. De verdikking van de INL zou kunnen komen door inflammatoire deposites of zeer subtiel intraretinaal oedeem, maar de verdunning van de IPL zou kunnen duiden op cel verlies en daarmee permanente schade.

De verdikking van de INL bevond zich meer in het centrale deel van het netvlies, terwijl de verdunning van de IPL zich meer aan de buitenste ringen van de ETDRS grid bevond. Het gebied dat met de OCT is onderzocht, is het (kleine) centrale deel van het netvlies. Met het ERG wordt de functie van het gehele netvlies gemeten. Als er dus ook een verdunning van de IPL in de perifere retina is, en alleen een centrale verdikking van de INL, kan dit inhouden dat deze verdunning een groter effect op het ERG heeft dan de verdikking van de INL.

Naast de verandering in de IPL en INL, zagen we ook verschillen in diktes in andere lagen: de retinal nerve fiber layer (RNFL), de ganglion cell layer (GCL), inner segment/outer segment layer (IS/OS), outer segment layer (OSL), en de outer segment photoreceptors/retinal pigment epithelium complex (OPR). Meestal waren de lagen dikker in ogen met een vertraagde b-golf, behalve de OPR, welke verdund was.

Deze bevindingen laten zien dat er een relatie is tussen een veranderde functie van het netvlies en veranderingen in de structuur van het netvlies. Welke van deze veranderingen eerder optreedt, en of een van beiden beter geschikt is in het opsporen van schade, moet in toekomstig onderzoek onderzocht worden.

Kinderuveïtis en jeugdreuma (JIA) geassocieerde uveïtis.

In de eerdere hoofdstukken werd gekeken naar volwassen patiënten met uveïtis. In **Hoofdstuk 5** onderzoeken we de ERGs van patiënten met niet-anterieure kinderuveïtis. Deze patiënten hadden een ERG gehad om een retinale dystrofie uit te sluiten. Retinale dystrofieën kunnen soms lijken op uveïtis, maar in tegenstelling tot uveïtis, is er helaas nog geen behandeling voor. Daarom is het van belang om ervoor te zorgen dat patiënten met een retinale dystrofie niet ten onrechte worden behandeld voor een uveïtis, en daarmee worden blootgesteld aan de mogelijke bijwerkingen van een voor hen niet effectieve behandeling. Daarnaast kan het van belang zijn voor zowel ouders als patiënten om op te hoogte te zijn van deze diagnose, omdat retinale dystrofiën erfelijk zijn.

Bij de patiënten met niet-anterieure kinderuveïtis was de b-golf ook vaak verlengd, maar vaak in combinatie met een afwijkende 30 Hz flicker respons. Deze ERG afwijkingen werden, net als bij de volwassenen, vaker gezien bij een ernstiger inflammatie. De ogen met een afwijkend ERG hadden vaker cystoïd macula oedeem (CME) (p = .021) en een hevige vitritis (3+ cellen) (p = .021). De visus was gelukkig nog wel goed (mediaan 0.05 LogMAR, IQR 0.00 - 0.15 LogMAR) en ook verschilde de visus niet tussen ogen met of zonder een afwijkend ERG. Dit houdt in dat bij kinderuveïtis de functie van het netvlies kan zijn aangedaan als de visus nog relatief goed is.

In **Hoofdstuk 6** beschrijven we het ERG van patiënten met jeugdreuma (JIA) geassocieerde uveïtis, een typische vorm van anterieure uveïtis. Bij acht patiënten was hier een ERG gemaakt, omdat er wat verdunning op de OCT te zien was. Van de 16 JIA uveïtis ogen, hadden er 10 afwijkingen op het ERG. Het type ERG afwijkingen verschilde. Er werden afwijkingen gezien in vrijwel alle aspecten van het ERG, behalve de latentietijden van de b-golf van het staaf ERG. De kegel b-golf was ook verlengd, maar dit was niet de meest voorkomende ERG afwijking.

Ogen met een actieve inflammatie hadden allemaal een afwijkend ERG, maar bij de JIA ogen die ten tijde van het ERG geen tekenen van inflammatie hadden, kon het ERG ook afwijkend zijn. Zelfs bij patiënten met een rustige uveïtis, zonder medicatie, kon het ERG afwijkend zijn. Dit duidt waarschijnlijk op permanente retinale schade, bij deze typische vorm van anterieure uveïtis.

Effecten van de ligging van de Dawson Trick Litzkow (DTL) electrode op het ERG.

In **Hoofdstuk 7** is gekeken naar de effecten van verschillende liggingen van de DTL electrode op het ERG. De DTL is de actieve electrode van het ERG. Het is een dun draadje dat tegen het oog aanligt. Soms kan de positie van dit draadje verschuiven tijdens de meting. Hierom wordt soms beweerd dat het beter is om de DTL niet onder de cornea langs het onderooglid (de lower lid position (LLP)) te plaatsen, maar in de onderste fornix (fornix position (FP)). De DTL zou op die plek minder snel verplaatsen. Een nadeel van de FP is dat de amplitudes er kleiner zijn, maar een groot voordeel zou zijn dat de ERG resultaten er minder variabel door worden.

In het UMC Utrecht wordt de LLP gebruikt voor het meten van ERGs. Mocht de FP inderdaad beter zijn, dan zouden we mogelijk onze lokale protocollen en referentiewaardes aan moeten passen. Hierom is bij de uveïtis patiënten het licht geadapteerde ERG twee keer gemeten: eerst in de LLP en daarna in de FP.

Tussen deze twee metingen waren er geen significante verschillen in de latentietijd. Dit houdt in dat de gevonden resultaten in hoofdstuk 2 - 6 niet significant beïnvloed kunnen zijn geweest door een tijdelijke verplaatsing van de DTL positie tijdens een ERG meting.

Wel werden er verschillen tussen de twee DTL posities in de amplitude gezien. Deze waren in de FP gemiddeld kleiner, maar de spreiding in amplitude was er ook proportioneel kleiner t.o.v. de LLP. De covariate of varience (CoV), ook wel bekend als de relatieve standaard deviatie, was namelijk niet significant verschillend tussen beide posities.

De spreiding van een normaalverdeling is dus ook verhoudingsgewijs kleiner in de FP. Dit houdt in dat de kleinere normaalverdeling van de FP geen valide reden

is om voor de FP te kiezen, deze is namelijk verhoudingsgewijs kleiner. Een voordeel van de FP is wel dat deze lokatie comfortabeler is voor patiënten. Een nadeel is dat bij aandoeningen waarbij de amplitudes heel klein zijn, in de FP sneller geen ERG meer gemeten kan worden, omdat de respons te klein is geworden, terwijl deze nog wel in de LLP te meten is.

Verder laat deze studie zien dat het belangrijk is om de positie van de DTL electrode te controleren tijdens het ERG. Als de positie veranderd is, is dit van belang bij de beoordeling van de amplitudes, maar niet bij de beoordeling van de latentietijden.

Samenvattend laat dit proefschrift zien dat in veel vormen van uveïtis de functie van het netvlies is aangedaan. Er is een karakteristieke afwijking op het ERG te zien in uveïtis: de vertraagde kegel b-golf, welke te maken heeft met de ernst van de ontsteking. Ook is er een relatie tussen een verminderde functie (een vertraagde kegel b-golf) en veranderingen in de structuur van het netvlies (een verdikte INL en een verdunde IPL).

Meestal zijn de ERG afwijkingen blijvend, maar gelukkig kan bij een deel van de patiënten het ERG verbeteren, wat inhoudt dat bij een deel van de patiënten het ERG nog omkeerbare schade kan detecteren. Toekomstig onderzoek moet uitwijzen of de vertraagde kegel b-golf hierom ook gebruikt kan worden in het monitoren van uveïtis.

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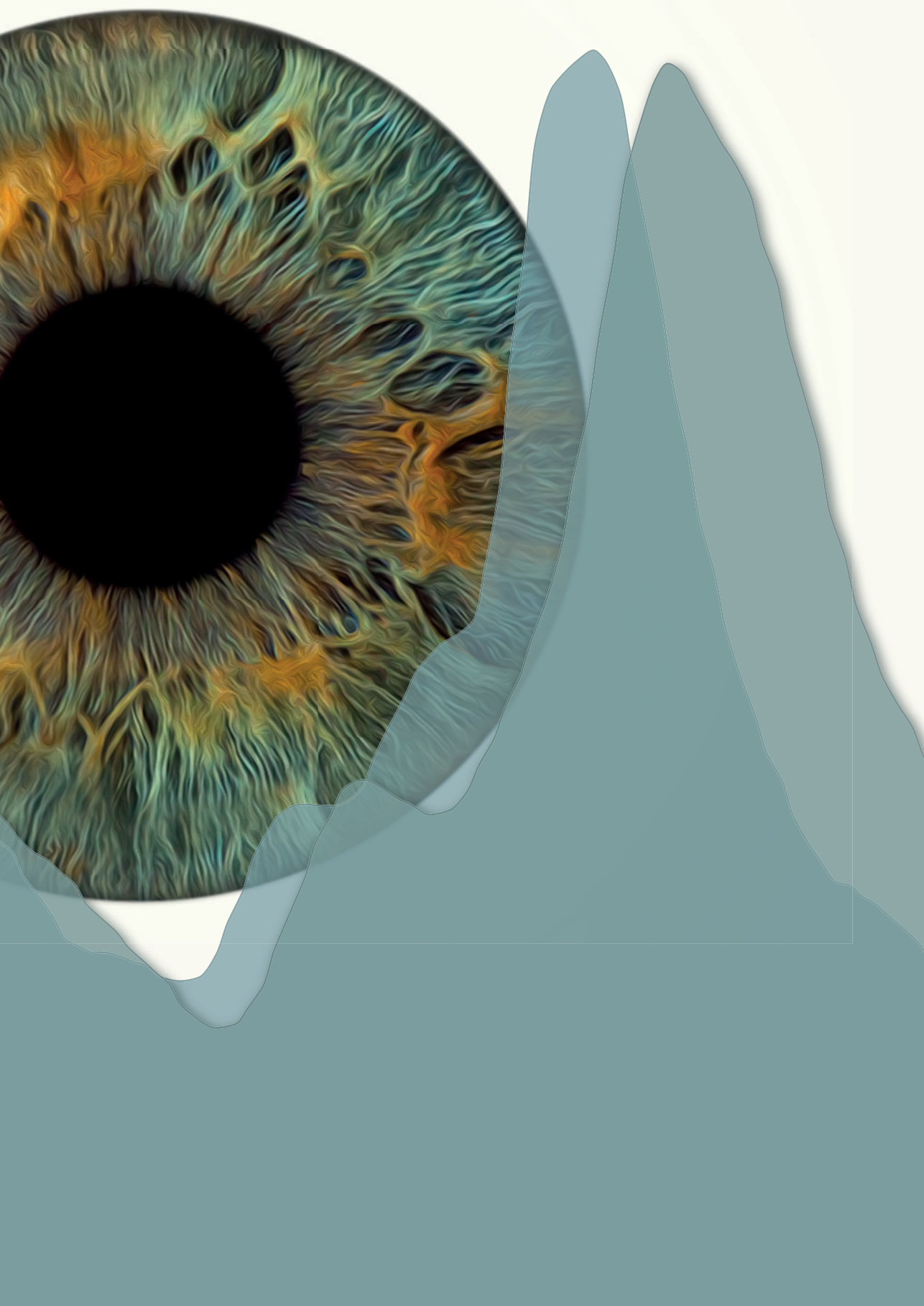
APPENDICES

DANKWOORD, ABOUT THE AUTHOR,
LIST OF PUBLICATIONS
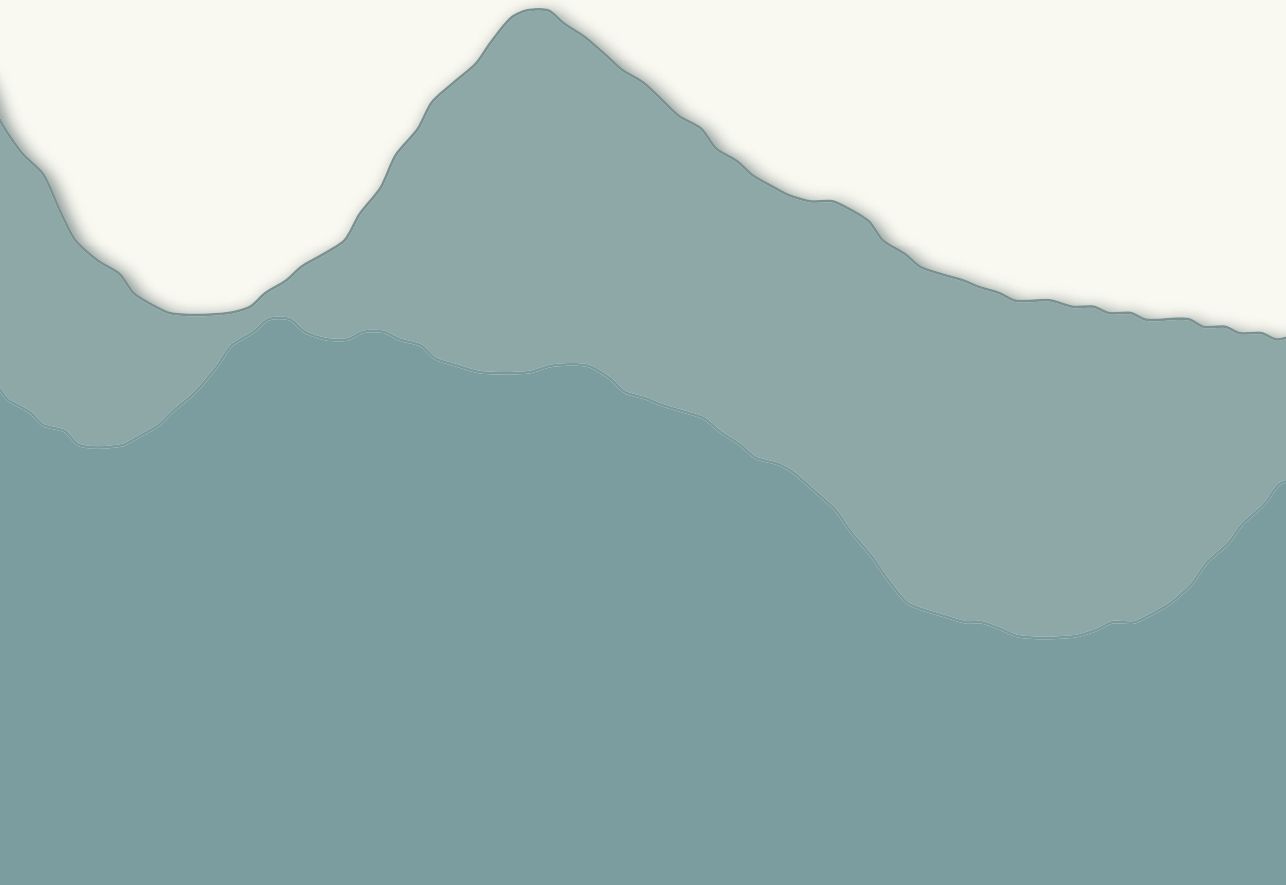
DANKWOORD

Dit proefschrift was nooit tot stand gekomen zonder de hulp van een grote groep mensen. Allereerst wil ik graag **alle patiënten** die aan onze onderzoeken hebben meegedaan bedanken. Zonder jullie participatie, hulp en enthousiasme was dit proefschrift nooit mogelijk geweest. Ik voel me vereerd dat jullie je tijd en energie hierin hebben willen steken en dat ik tot tweemaal toe onze resultaten aan jullie heb mogen presenteren op het Oogcongres.

Daarnaast wil ik graag een aantal personen in het bijzonder bedanken. Allereerst mijn promotores. De wens van jullie, Joke en Mies, om jullie expertises te combineren om zo meer inzicht te krijgen in de effecten van uveitis op de functie van het netvlies heeft tot dit promotieonderzoek geleid.

Joke, jij stond aan de wieg van mijn wetenschappelijke carrière. Jouw motiverende stagebegeleiding heeft mijn interesse in de wetenschap en uveitis gewekt. Ik ben heel blij dat ik jou en Fleurieke destijds als begeleiders heb getroffen. Jouw enthousiasme, je doortastendheid en betrokkenheid hebben mij ook intens geholpen en gesteund tijdens mijn promotie. Ik heb veel geleerd van jouw schat aan kennis over de uveitis, en heel veel gehad aan jouw constructieve feedback tijdens het schrijven van wetenschappelijke artikelen. Dank voor je geduld, tijdens o.a. het scoren van de vele FAGs, maar bovenal voor je vertrouwen in mij en in dit onderzoek. Zonder jou was mijn promotietraject nooit gelukt. Ontzettend bedankt hiervoor!

Mies, jij en het team in Bartiméus hebben mij enorm veel geleerd over de elektrofysiologie. Ik bewonder je tomeloze kennis over de oogheelkunde en de elektrofysiologie. Jouw kritische vragen en opmerkingen hebben menig artikel naar een hoger niveau getrokken. Jouw kunde in het herkennen van patronen in het ERG heeft mij enorm geholpen bij de interpretatie van de resultaten en hebben het onderzoek richting gegeven. Daarnaast heeft jouw scherpe blik en perfectionisme de vele presentaties die ik heb mogen geven eindeloos verbeterd. Enorm bedankt!

Gerard, jouw technische benadering van de elektrofysiologie was erg waardevol tijdens deze promotie. Je handige softwareprogramma Diagnostic tools heeft de interpretatie van ERGs een stuk inzichtelijker gemaakt en mijn leven een stuk makkelijker gemaakt doordat hiermee het verzamelen van data veel makkelijker werd. Je kritische blik op de vele statistische analyses hebben mij, en het hele team aan het denken gezet en het eindresultaat uiteindelijk een stuk beter gemaakt. Dank hiervoor!

Commissieleden: **prof. dr. B. Oldenburg, prof. dr. R.L.A.W. Bleys, prof. dr. A. Rothova, prof. dr. N.M. Jansonius en dr. M. Schooneveld** enorm bedankt dat jullie de tijd wilden nemen om mijn proefschrift te lezen en te beoordelen.

Mijn paranimfen, Sara en Pieter, enorm bedankt voor jullie steun op de momenten dat het tegenzat tijdens dit project. **Sara**, jij werd al snel mijn kamergenootje en we hebben samen als 'de Q-dames' lief en leed gedeeld. Dank voor je altijd luisterende oor en je kunst tot relativeren als het even niet zo lekker ging. Jouw enthousiasme over R heeft, ondanks de frustraties die dit fantastische programma ook regelmatig kan geven, mijn wetenschappelijke leven uiteindelijk een stuk makkelijker, en vele malen beter gemaakt. Ik ben nog altijd blij dat ik de switch van SPSS naar R heb gemaakt. Daarnaast stimuleert en motiveert jouw enthousiasme voor de wetenschap mij enorm. Maar ook op privé vlak heb je een wereld voor mij geopend: menig vegan gerecht is inmiddels ook bij ons thuis met veel succes geïntroduceerd.

     Pieter, mijn schat, mijn rots in de branding. Dank voor je steun, voor je rust, voor je eindeloze knuffels, en voor je liefde. Onze promotietrajecten hebben we tegelijkertijd doorlopen en daardoor weet jij als geen andere echtgenoot wat promoveren inhoudt. Je herkent de vele hobbels en weet ook welke dingen het leven als promovendus een stuk makkelijker (en moeilijker) maken. Dank voor als je liefdevolle uitleg over de - soms erg ongebruiksvriendelijke - Adobe applicaties. Zonder jouw hulp waren de illustraties van mijn artikelen een stuk minder mooi geworden; om over de lay-out van dit boekje maar te zwijgen. Dank je wel, dat je me wetenschappelijk, maar ook persoonlijk inspireert en motiveert. Samen hebben we ons op het onderzoek gestort en samen hebben we ons ook intens verheugd op wat (niet) zou komen na het boekje. Onze huwelijks-droomreis mag dan een tijdje in de vriezer gaan, maar ik ben nog altijd blij dat ik met je ben getrouwd!

Collega (oud)onderzoekers enorm bedankt voor het warme en gezellige team waarin ik heb mogen samenwerken. Dank voor alle steun, tips en jullie waardering voor mijn baksels.

     Fleurieke: jij hebt als begeleider van mijn masteronderzoek mijn interesse voor de wetenschap aangewakkerd. Je hebt mij in korte tijd, door je eindeloze aandacht, enthousiasme en tips zo ontzettend veel geleerd: van databases opzetten, tot analyseren en schrijven. Jouw begeleiding heeft mij op het juiste spoor gezet om te beginnen aan dit wetenschappelijke avontuur. Enorm bedankt daarvoor!

     Anne-Mieke, Nienke, Laura, Yvonne, Daniel, Kamil en Ymkje, dank voor jullie warme onthaal, gezelligheid en de vele tips toen ik als onderzoeker begon. Ik kijk er naar uit om als AIOS weer jullie collega te zijn. Kamil: dank voor je voor al

je tips die menige brief en aanvraag van mij hebben verbeterd. **Roos, Bas, Evianne, Myrthe, Wouter en Brandon**, dank voor de gezelligheid van het B-hok, en voor de vele koffietjes en taarten die we samen hebben genuttigd. **Lude, Carlijn, Deniz, Els en Jeanneau** jullie zijn aan het einde van mijn traject collega's geworden: dank voor de gezelligheid en veel succes met jullie promoties!

Het uveitis team: **Ninette, Ralph, Annette, Lintje, Viera en Maartje**: enorm bedankt voor jullie enthousiasme en jullie hulp met het includeren van patiënten. Zonder jullie was mijn promotie nooit gelukt. Bedankt voor de leerzame uveitis besprekingen en jullie gezelligheid tijdens de congressen en eilanddagen. Ninette en Ralph, enorm bedankt voor het warme onthaal bij jullie uveitis poli's. De dagen dat ik met jullie mee mocht draaien waren enorm waardevol voor mij. Dank voor jullie geduld, gezelligheid, enthousiasme en uitleg over uveitis. **Ina en Ralph**: bedankt voor jullie hulp met het includeren van patiënten als ik er niet kon zijn. **Jonas**, bedankt voor je open deur, en je frisse blik op mijn projecten.

Collega's van Bartiméus, dank voor jullie warme ontvangst en geduldige uitleg over alles in de elektrofysiologie. Dank voor jullie kennis en enthousiasme tijdens menige bespreking, maar ook voor de gezelligheid tijdens de ISCEV en BriSCEV congressen. Jullie hebben me er echt bij laten voelen. **Herman**, bedankt voor de vele nuttige discussies en jouw hulp met het uitvoeren van zo veel experimenten!

Alle stafartsen en AIOS van het UMCU: bedankt voor jullie interesse en hulp tijdens mijn promotie. Ik voel me vereerd dat ik de opleiding tot oogarts bij jullie mag volgen en kijk er enorm naar uit om jullie team te komen versterken!

Josianne en Fahriye, dank voor jullie heerlijke gezelligheid en discussies tijdens de eilanddagen en andere uveitis congressen. Ik kijk er naar uit om jullie nog vaak tegen te komen in AIOS land!

Carola, Hanane, Vincent, Bram en Mahfam, dank voor jullie inzet en enthousiasme als wetenschapsstudenten. Jullie mogen begeleiden was voor mij erg leerzaam en ik kijk er naar uit om jullie in de toekomst weer als collega's te treffen. Veel succes gewenst in jullie carrières! Vincent, bedankt voor je enorme inzet in het OCT stuk, de vele discussies en de gezelligheid, ik vind het erg leuk dat we binnenkort collega's worden.

De **TOA's**: en dan in het bijzonder **Dagmar, Yvonne, Jane en Marjolein**, bedankt voor al jullie hulp bij het meten van menig ERG en voor al jullie tips en truucjes rondom het meten van een ERG, maar bovenal bedankt voor alle gezelligheid in het OCT/ERG hok. **Lotte**, dank voor alle gezellige katten praatjes (Floortje is nog steeds blij met alle speeltjes tips) hierdoor werden de ERG metingen tijdens de lunch een stuk leuker! **Jos, Irene en Hilda** dank voor jullie hulp met het opsporen van oude FAG opnames.

Secretaresses, enorm bedankt voor al jullie hulp bij het inplannen van de vele ERG onderzoeken. In het bijzonder wil ik **Brigitte** bedanken: jouw interesse voor mijn onderzoek en je inzet om altijd maar een gaatje te vinden in de drukke agenda's was een enorme steun in de vaak lastige logistieke puzzel! **Suzan en Petra:** bedankt voor jullie hulp met het logistieke doolhof van briefpapier, enveloppen, stickers etc. Suzan, enorm bedankt voor je hulp met het opsporen van de juiste uveitis patiënten via SAS.

Het onderzoeksteam: **Tamara, Marianne en Renate**: bedankt voor jullie hulp en uitleg over het doolhof van regeltjes waar we ons als onderzoekers doorheen moeten manoeuvreren. Tamara in het bijzonder: dank voor al je snelle reacties op mijn vele METC aanvragen en je open deur.

De ICTers en technici: **Ferry, Ruud en Arjen**, dank voor jullie hulp en uitleg bij de vele ICT-vraagstukken die tijdens mijn promotie opdoken. **Peter Zuidhof**: dank voor je statistische adviezen.

Familie en vrienden, dank voor jullie gezelligheid en steun tijdens mijn promotietijd. De mensen om mij heen geven het leven kleur en maken alles zoveel leuker en fijner! Dank voor alle gezelligheid tijdens de vele borrels, etentjes, uitjes en voor jullie enthousiasme over mijn kook- en bakkunsten. Dit was een heerlijke manier voor mij om soms op iets totaal anders te kunnen focussen tijdens mijn promotie traject. Enorm bedankt voor jullie luisterende oor en steun als het weer eens tegenzat.

Pap en mam, jullie hebben mij van jongs af aan gestimuleerd. Zonder jullie steun en advies, was ik nooit gekomen waar ik nu ben. Dank voor jullie vertrouwen in mij, en in dit proefschrift.

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ABOUT THE AUTHOR

Anna Henriette Brouwer was born on February 9th 1991 in Amsterdam. After graduating from high school in 2009 from the Montessori Lyceum Amsterdam, she moved to Utrecht to study medicine at the University Utrecht.

Her master thesis "Potential Predictors of Poor Visual Outcome in Human Leukocyte Antigen-B27-Associated Uveitis." resulted in a publication in the American Journal of Ophthalmology.

In December 2015 she received her medical degree and started her PhD research in 2016 under the supervision of prof. dr. J.H. de Boer, prof. dr. M.M. van Genderen and dr. G.C de Wit. During her PhD candidacy she was awarded the 'best oral presentation' grant at the Dutch Ohthalmology PhD Students (DOPS) conference in 2018, and received an international Association for Research in Vision and Opthamology (ARVO) travel grant to speak at the ARVO conference 2019 in Vancouver. Twice she was an invited speaker at the Dutch ophthalmology patients' conference 'Oogconges'.

Anna enjoys to travel, cook, and loves to bake. During her study she cofounded a small baking company 'Nana's Pastries', with her friend Narda. Currently she only bakes for family and friends.

Anna lives together with her husband Pieter and their cat Floortje in Utrecht, the Netherlands. They own a Toyota Landcruiser "Teddy" with which they planned to tour the Middle-East and Africa after finishing both their theses. Due to the coronavirus pandemic these plans were postponed indefinitely. Anna started her residency in ophthalmology at the University Medical Center Utrecht in September 2020.

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