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Evaluation of the visual pathway with ERG, mfERG and mfVEP in inherited eye disorders

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To my mother

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ABBREVATIONS

CDI	Color doppler measurements
CRA	Central retinal artery
CRT	Cathode-ray tube
DOA	Dominant optic atrophy
EDV	End diastolic velocity
ERG	Electroretinography
IR	Infra red
LGN	Lateral geniculate nucleus
LHON	Leber`s hereditary optic neuropathy
mfERG	Multifocal electroretinography
mfVEP	Multifocal visual evoked potential
PERG	Pattern electroretinography
PSV	Peak systolic velocity
RI	Resistive index
RP	Retinitis pigmentosa
SLDF	Scanning laser doppler flowmetry
SNR	Signal-to-noise ratio
VEP	Visual evoked potential

INTRODUCTION

General background

Inherited disorders of the retina and of the central visual pathway constitute a considerable group of various eye disorders. During the past ten years several genetic defects have been identified, in a number of proteins that are essential for visual function. Further knowledge of the pathogenesis of these disorders and identification of the visual dysfunction in these patients are needed. In addition to the standard ophthalmological examination different electrophysiological methods are valuable, offering objective assessment of function which is essential for correct diagnostics. These methods are of special importance when the patient presents with minimal fundus pathology or if the patient fails to cooperate in our routine ophthalmological examinations.

Already as early as 1865 the first electroretinogram (ERG) was performed by a Swedish ophthalmologist, Fritjof Holmgren in Uppsala, who measured the retinal responses to light in a frog (Holmgren 1865). Further development of the electrophysiological methods made it possible to analyse the response from the human retina which later on became an important diagnostic tool for diseases affecting retinal function. The clinical use of ERG has been continuously increasing, and the methods have been modified and adjusted in order to give additive and more specific information of retinal function. These methods have considerably improved our knowledge of different retinal disorders. Some advances of special importance in retinal electrophysiology are the separation of the rod-cone response (Gouras 1970), the identification of the small residual response when retinal function is reduced with more than 98 % (Berson et al. 1985) and the distinction of stationary retinal disorders from generalised retinal degenerations (Berson et al. 1969).

The full-field ERG is a reliable method for measuring responses from rods and cones throughout the entire retina which is clinically an invaluable help in differentiating tapetoretinal degenerations even in some central retinal degenerations (Niemeyer et al. 1983). However, it does not give any specific information of localised dysfunction in the macular region. Recent developments in ERG technology has made it possible to obtain responses from localized areas of the retina, specifically from the macular region. A few years ago the

most widespread method used was the Doran Focal ERG, developed for obtaining the cone ERG from the macular region (Sandberg et al. 1979), which gave an estimate of central macular function. Another method for evaluating the function in the macula and the inner retina is the pattern electroretinogram (PERG) (Holder 2001; Robson et al. 2003), which is used both clinically and in research. However, during recent years, the multifocal electroretinogram (mfERG) developed by Erich Sutter (Sutter et al 1992), has become the most valuable method for identifying dysfunction in localized areas in the central part of the retina.

Further developments of the mfERG technique have been the introduction of the multifocal visual evoked potential (mfVEP) (Baseler et al. 1994). Also in the mfVEP a limited area of the central retina is stimulated, but instead of assessing the response from the retina, the cortical responses are obtained using cortically placed electrodes. This new and objective technique has given us further insight in the function of the visual pathway, which certainly improves our ability to identify dysfunction in different regions of the visual pathway.

Another field of major importance for evaluating inherited eye disorders has been the development in molecular genetics. Since 1990, when Thaddeus Dryja identified the mutation in the rhodopsin gene in families with retinitis pigmentosa (Dryja 1990) we have achieved increasing knowledge of the pathogenesis behind hereditary retinal degenerations. Today we know that several hundred genes have been identified, which are involved in the development of inherited eye disorders. One step towards the treatment of these disorders is combining a highly specified knowledge of visual pathway dysfunction with specific analyses of the associated protein defect. This phenotype-genotype correlation may be a prerequisite for gene therapy in the future. This knowledge is already essential for clinical competence in diagnosing and reliably estimating the prognosis in this markedly variable group of diseases that constitute inherited eye disorders.

The central visual path way

Vision is generated by the electric activity in the different cell layers of the retina and along the optic tract to the visual cortex. After an initial absorption of light in the photoreceptors the light stimuli reach the pigment epithelium. The electric activity in the retinal cells depends on several environmental factors such as light- or dark adaptation, the wavelength and the frequency of the incoming light. All these factors are related to the responses assessed by the ERG (Berson 1993).

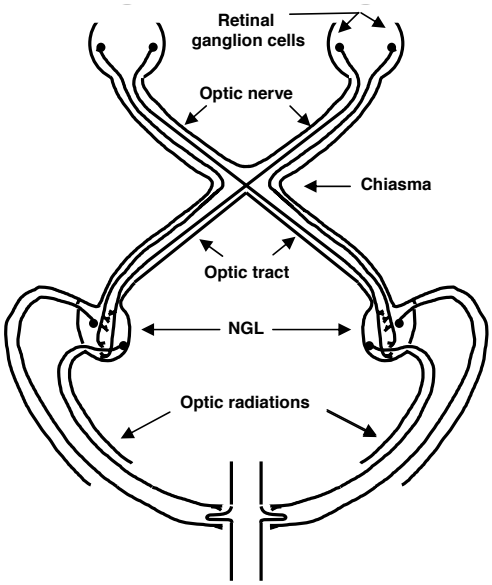

Figure 1. *An overview of the visual pathway with cross over of the nasal ganglioncells in the chiasma.*

The activity in the optic nerve and in the optic tract leading to the visual cortex is complicated and difficult to fully understand. Simplified, the vision impulse leaves the eye by the retinal ganglion cells in the optic nerve. In the chiasma there is a partial crossing of the axons from the nasal retina corresponding to the temporal visual field, which is of clinical importance and therefore relevant to investigate. After the chiasma, the axons from the temporal retina join together with the axons from the nasal retina in the contra lateral eye, in the optic tract.

The optic tract wraps around the midbrain towards the lateral geniculate nucleus (LGN), where all the axons form synapses. Then they fan out through the deep white matter of the brain forming the optic radiations, which will ultimately reach the primary visual cortex (area V1), located at the back of the brain (Jeffery 2001). Measuring activity from the visual cortex itself will thus allow an objective assessment of the quantity of the transmitted signal that actually reaches its target, and thereby the quality of the image that we perceive.

Electrophysiological examinations

Full-field electroretinography (ERG)

The electroretinogram is the sum of action potentials recorded with a bipolar contact lens on the cornea, when the retina is stimulated with light. The entire retina is stimulated in a full-field Ganzfeld screen, all retinal cells are thereby simultaneously exposed to the light stimulus. The responses are believed to have a different origin within the retina, the a-wave deriving mainly from the photoreceptors and the b-wave mainly from the Müller cells and the bipolar cells (Granit 1947; Bush et al. 1994). With different light filters and light intensities it is possible to separate the responses from cones and rods (Gouras 1970). When the eye is fully dark adapted and stimulated with a dim blue light the response reflects mainly the rod function. A single white light is used to stimulate the dark adapted eye, which demonstrates the combined rod and cone response reflecting the total retinal function. With 30 Hz flickering white light the selective cone response is assessed. The maximum amplitude of the response is measured in microvolt (μV) and the implicit time (time from stimulation to maximum response) is measured in millisecond (ms).

For analyzing a severely reduced remaining retinal function (less than 1,0 % of the normal response), a special technique has been developed. By combining a full-field stimulation and an analogue or a digital narrow band pass filter with computer averaging, it is possible to extend the lower amplitude limit down to 0.05 μV (Berson et al. 1985).

Multifocal-electroretinography (mfERG)

The density and nature of the cones change dramatically with retinal eccentricity from the fovea towards the periphery (Österberg 1935; Curcio et al. 1990). As a result, the full-field cone ERG is a mixture of responses from a diverse array of cells and from different retinal regions. The development of the multifocal technique (Sutter et al. 1992) has made it possible to evaluate local responses, from the central retina corresponding to approximately 25° of the central visual field. This central part of the retina includes approximately 23% of the human cone receptors, and the densities of the bipolar cells are roughly proportional to the cone density (Curcio et al. 1990).

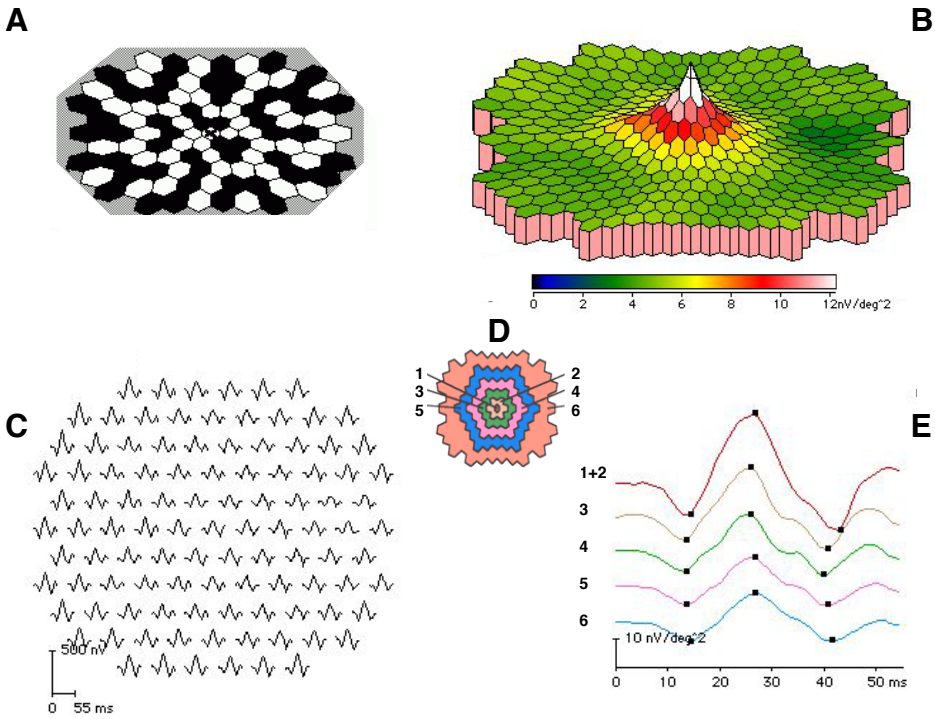

Figure 2. *A normal mfERG, first-order kernel. (A) The stimulating picture. (B) The plot response, retinal view. (C) The traces, retinal view. (D) The rings. (E) The averages.*

During the examination the patient looks intensely at a pattern of 103 (61-241) black and white hexagons on a presenting CRT monitor or CRT display in an IR camera. The camera section of the IR camera is used to control the fixation. To produce focal responses of approximately equal amplitudes the sizes of the hexagons are scaled, the hexagons increasing in size with increasing distance from the centre (Sutter et al. 1992). Each hexagon goes through a pseudo-random sequence, the m-sequence, of black and white presentations, frame changes. Most frequently used is a frame rate of 75 Hz which means that the frame is changed every 13.33 ms. Every hexagon stimulates the focal part of the retina with the same m-sequence of white and black presentations, but each hexagon starts at a different point in the sequence. As in full-field ERG a bipolar contact lens is used to record the responses. During the recording the response to a single hexagon is built up while the responses associated with all other hexagons are eliminated by using an advanced algorithm.

The mfERG responses can be assessed from different kernels. The first-order kernel responses are believed to have mainly the same origin as the full-field cone ERG response (Sutter et al.

1992; Nagatomo 1998; Verdon et al 1998; Hood 2000; Marmor el al. 2003). Diseases of the photoreceptors or of the bipolar cells result in small and reduced amplitudes, but a normal to slightly delayed implicit time (Hood 2000). There is however described some contribution from the inner retina and the ganglion cell layer (Sutter et al. 1999, Hood 2000, Kurtenbach et al. 2004). This contribution has been demonstrated in the first-order kernel especially when modifying the settings by lowering the stimuls frequency or reducing the contrast but the contribution seems to be more pronounced in the second order kernel. A non-linear component which is also more pronounced in the second order kernel has been described by Sutter and Bearse as an optic nerve head component. This component is believed to have its origin in the ganglion cell axons (Sutter et al. 1999) and may explain the naso-temporal variation of the responses showing increased latencies with increased eccentricity to the optic nerve head. By studying the second-order kernel response, this component can be analyzed, which would make it possible to evaluate also the ganglion cells. The contribution from the inner retina and the ganglion cell layers is still discussed and needs to be investigated more extensively.

Multifocal Visual Evoked Potential (mfVEP)

The visual evoked potential (VEP) has been used clinically for many years to study the retino-cortical pathways (Beauchamp et al 1976, Odom et al. 2004). The responses from the standard VEP reflect the total response from the visual cortex to stimulated area in the retina at a time and the method does not offer a topographical description of the injured visual field. With the full-field VEP the response is mainly generated from the upper retino-cortical pathway and with a central overrepresentation because of the anatomy of the cortex (Horton et al 1991). This means that lesions in the visual pathway generated from the lower retina and the periphery can be drowned in the total VEP response. Studies have also shown that standard VEP responses may have a large contribution from the extrastriate cortex (Di Russo et al. 2002). By developing the multifocal technique described above (Sutter et al. 1992), some of these problems have been overcome. The multifocal visual evoked potential (mfVEP) reflects the cortical activity topographically corresponding to the central visual field (Baseler et al 1994 and 1997). Localized lesions in the central visual field can therefore consequently be identified (Klistorner et al. 1998; Betsuin et al. 2001; Goldberg et al 2002). The mfVEP is

described to be mainly generated in the primary visual area, V1, with very little contribution from the extrastriate cortex because of the high frequency stimulation (Slotnick et al. 1999; Hood et al. 2003).

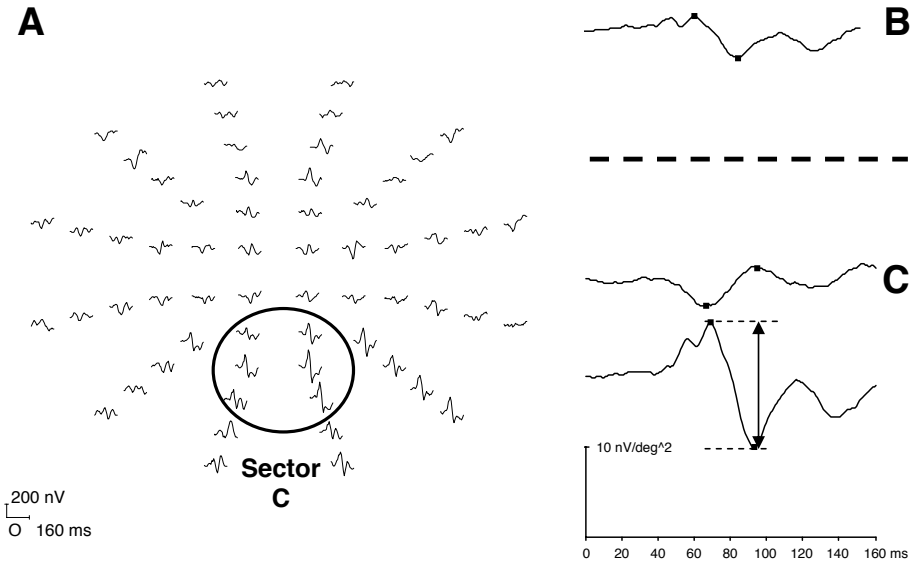

Figure 3. *A normal mfVEP. (A) The traces, demonstrating the corresponding cortical response to the central visual field. The ring presents sector C, the region in the mfVEP where the highest amplitudes are measured during the midline electrode position. There is a different polarity in the amplitudes from the upper hemi field compared to the lower hemi field. (B) The average from the summed mfVEP traces. (C) The averages from the summed mfVEP traces for the upper hemi field respectively the lower hemi field. The difference in polarity and in maximum response is more clarified, as why the summed response in B is so low. The arrow indicates the maximum response from the first two components, which correlates to N 70 and P 100 in the classic VEP.*

During a mfVEP examination the patient looks at a dartboard pattern on a CRT screen (fig. 6). The pattern is divided in to 60 sectors containing 8 white and 8 black checks each. The array is cortically scaled to provide 60 mainly equal sized responses. The pattern contrast reverses in each sector and is modulated according to the same m-sequence as described above (Sutter et al. 1992). The responses are recorded in the standard examination with two mid line scalp electrodes over the occipital cortex and with the ground electrode behind one ear or on the forehead.

As a result of this bipolar registration and the cortical anatomy, the responses have opposite polarity in the upper hemi field compared to the lower hemi field (fig. 3). The recommended

bipolar registration presents reduced responses in the central horizontal line (Klistorner et al. 1998) which can be avoided by using multiple channel registration (Klistorner et al. 2000; Hood et al. 2003).

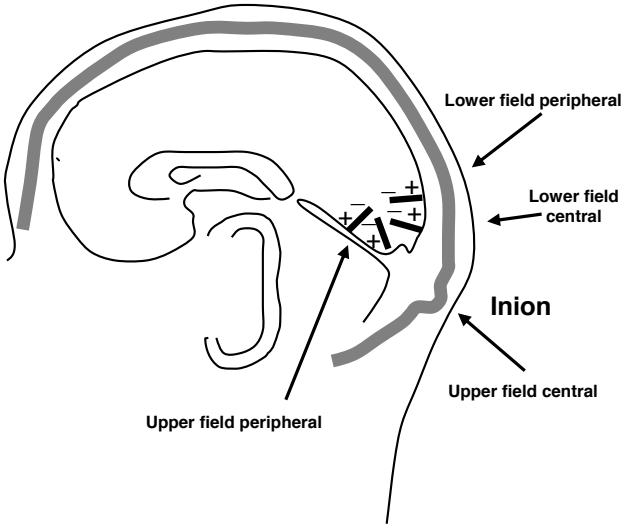

Figure 4. *Schematic representation of the relative positions and orientations of the visual field.*

Further the responses from the upper hemifield demonstrate reduced amplitudes compared to the lower hemi field (fig. 3). This reduction of the responses from the upper visual field is also described for the traditional VEP when stimulating in different parts of the visual field (Halliday et al. 1977), which is explained by the cortical representation of the visual field (fig. 4).

Because of the inter-individual variability of the mfVEP responses an inter-ocular comparison of the responses has been introduced by Hood et al. in order to increase the sensitivity and reliability of the method (Hood et al. 2000). However, when using this kind of method only unilateral lesions anterior to the chiasma can be detected (Bengtsson et al. 2005).

Further evaluation of responses from specified areas, including responses with the same polarity giving rise to extremely large amplitudes with a high signal to noise ratio (SNR), may be of major importance for interpretation of the cortical response. A further improvement may be to compare these localized responses from both hemispheres which is possible to do by using a two registration channel system.

This thesis will describe the usefulness of the mfVEP in some hereditary eye disorders and the modification of the technique for improved interpretation and clinical value of the method.

AIMS OF THE STUDY

General aims

- To examine and describe the clinical phenotypes with emphasis on electrophysiology, in patients with different hereditary eye diseases.
- To further evaluate and modify the mfVEP technique for clinical use.

Specific aims

Paper I:

Bothnia Dystrophy is a tapetoretinal disorder with a mutation in the RLBP1 gene. The aim was to investigate the phenotype in two young patients with this disease and in one of the patients further study the specific alteration in the rod function and evaluate the macular function with mfERG.

Paper II:

A family with dominant optic atrophy and a known mutation in the OPA-1 gene was examined with ERG, mfERG, mfVEP and ocular blood flow measurements. The aim was to evaluate these new methods for improved identification and characterization of this disorder.

Paper III:

The aim was to examine with ERG, mfERG and mfVEP, a cohort of patients with retinitis pigmentosa, who still had remaining central visual fields, and who had been followed during a period of at least 7 years at the department of Ophthalmology in Lund.

Paper IV:

MfVEP, which is a relatively new method developed from the mfERG, demonstrates the cortical response corresponding to the central visual field. The aim was to modify this method in order to improve the clinical value. By using an IR-camera for stimulation and for controlling the fixation, and by introducing a two channel system, the aim was to find out

whether the visual pathways can be additively analyzed regarding the inter-ocular difference and the uncrossed/crossed visual pathway.

Paper V:

Leber`s hereditary optic neuropathy (LHON) is a hereditary eye disorder. The aim was to follow the acute phase of the disease with mfVEP in a patient with a known mutation for LHON.

METHODS

Ophthalmological examination

Ophthalmological examination included assessment of best corrected visual acuity, slit lamp inspection, ophthalmoscopy, fundus photography and in one patient evaluation of pupillary function.

Kinetic perimetry was performed with a Goldman perimeter using targets I_{4e} and V_{4e} and in paper II and V also targets 0_{2e} and 0_{3e} for detecting central visual field defects.

In paper II the color vision was tested with the D-15 Farnsworth method.

In paper I the dark adaptation thresholds for the rods were obtained with a Goldman-Weeker adaptometer after the standardized 40 minutes of dark adaptation, and after a prolonged dark adaptation (20-24 h). The research procedures were in accordance with institutional guidelines and the Declaration of Helsinki.

Electrophysiological examinations

Full-field electroretinography (ERG)

ERGs were recorded in a Nicolet Viking analysis system (Nicolet Biomedical Instruments, Madison, WI) and as previously described by Andréasson et al (Andréasson et al. 1997). In the eye tested the pupil was maximal dilated with topical 10% phenylephrine hydrochloride and 1% cyclopentolate hydrochloride. Standardized dark adaptation is 40 minutes but in paper I the reexamined patient was exposed to a dark adaptation that was prolonged to 24 h. respectively 20 h. A Burian-Allen bipolar contact lens was applied on the topically anesthetized cornea together with a ground electrode on the forehead. The different light stimuli were reflected from the Ganzfeld sphere (Grasby Optronics, 350 liniar/log optometer, Lumilens model 1153).

The light stimuli deviated from ISCEV standards (Marmor et al. 2004) by using a weaker flash (0.8 cd s/m^2) than the standard flash (1,5-3,0 cd s/m^2). Responses were obtained with a

wideband filter (-3 dB at 1Hz and 500 Hz). Stimulation was with single full-field flashes (30µs) of dim blue light (Wratten filters #47,47A, and 47B) and white light (0.8 cd s/m^2). If recorded responses measured less than 10μV with single white flashes, recordings were also obtained with computer averaging (30 flashes), a bipolar artefact rejecter and a line frequency notch filter (50 Hz). Cone responses were obtained with 30-Hz flickering white light (0.8 cd s/m^2) averaged from 20 sweeps. To obtain small residual cone responses, as in the RP patients, the stimulation also included 200 flashes of flickering white light (30 Hz) and a digital narrow band pass filter tuned to 30 Hz (Andréasson et al. 1988). Results of the full-field ERG responses were compared with normal values obtained from 70 examined subjects with no signs of eye disease.

Multifocal-electroretinography (mfERG)

MfERGs were recorded using the Visual Evoked Response Imaging System (VERIS) (EDI. San Mateo, CA), developed by Sutter et al. (Sutter et al. 1992; Bearse et al. 1996). After maximal dilation of the pupil with topical 10% phenylephrine hydrochloride and 1% cyclopentolate hydrochloride the cornea was topical anesthetized and a gold or a Burian-Allen bipolar contact lens was applied to the ocular surface and a ground electrode to the forehead. The stimulus matrix consisted of 103 hexagonal elements that were displayed on a CRT monitor, in paper I and II, and on a CRT display in an IR camera, in the other papers. Both were driven at 75 Hz frame rate. The sizes of the hexagons were scaled with eccentricity according to the cone density to elicit approximately equal amplitude responses at all locations. At a viewing distance of 27-40 cm to CRT monitor and of 5 cm to the CRT screen in the IR camera the radius of the stimulus array subtended approximately 20-25 degrees. The luminance of each hexagon was independently alternated between black and white according to a pseudorandom binary m-sequence at 75 Hz. The maximum luminance was 138.0 cd/m^2 and the minimum luminance was 3.5 cd/m^2, resulting in a mean luminance of approximately of 70.8 cd/m^2. The responses were passed through a band-pass filter between 10 and 300 Hz. A small black fixation object was placed at the center of the stimulus matrix. To control the fixation a separate video system was used together with the CRT monitor and when using the IR camera the camera part of the IR camera was used for this purpose. Then the optical correction was made in the lens system of the camera for each patient.

Multifocal Visual Evoked Potential (mfVEP)

MfVEP was recorded using the same VERIS-system (EDI San Mateo, CA) developed by Sutter et al, as described for recording mfERG (Baseler et al. 1994 and 1997). In paper II the visual stimuli was produced on a CRT monitor while it was displayed on the CRT screen in the IR camera in the following papers. The stimulating image had the appearance of a dartboard containing 60 segments. These segments were cortically scaled in order to produce 60 recordings of approximately similar amplitude from the visual cortex. Each segment contained a checkerboard pattern with 16 checks, 8 white (138.0 cd/m^2) and 8 black (3.5 cd/m^2) that contrast reversed in a pseudorandom binary m-sequence at 75 Hz. The signals were amplified 100 000 times and were passed through a band-pass filter between 3 and 100 Hz. Each run contained 16 segments a 27 seconds with a total recording time of 7,2 min.

At a viewing distance of 40 cm when using the CRT monitor and 5 cm when using the IR camera the radius of the stimulus array subtended approximately 20-25 degrees.

All our recordings were bipolar but with little difference in electrode positions. Initially, in paper II, the electrodes were placed 2 cm above and 2 cm below the inion, in accordance with the electrode positions used by Klistorner et al. (Klistorner et al. 1998) to minimize the response difference from the upper and the lower cortical hemifields. In the remaining papers the lowest electrode was placed at the inion as described by Hood et al. (Hood et al. 2003) and the other one 4 cm above the inion. These electrode positions evoked higher differences between the amplitudes from the upper and lower hemi fields but there was much less muscle disturbance in the responses.

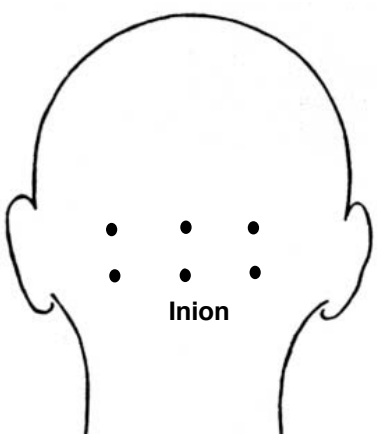

Figure 5. *Electrode positioning. The midline electrodes are placed on the inion and 4 cm above the inion. The lateral electrodes are placed 4 cm to the left and 4 cm to the right of the bipolar midline electrode position.*

In paper IV a two channel recording system was used. Two other electrode positions were added to the usual mid line position for recording from different cortical areas during the same stimulating run. The other two pairs were distributed 4 cm to the left and 4 cm to the right of the first pair of electrodes (fig. 5). The ground electrode was placed behind the right ear in all recordings.

When using the CRT monitor the fixation was controlled by a camera mounted close to the monitor while the fixation was controlled by the camera part of the IR camera when the IR camera was used for examination.
During all recordings the subjects were seated comfortably, to minimize muscle interactions, fixating the center of the dartboard. The pupils were not dilated and the stimulation was monocular with the contra lateral eye carefully occluded. A dim room light was used as background illumination.

Ocular blood flow measurements

Blood flow measurements

Three of the patients in paper II were examined with blood flow measurements, the scanning laser Doppler flowmetry directly preceding color Doppler imaging, at the Department of Ophthalmology and at the Department of Clinical Physiology in Malmö.
Intraocular pressure was measured within one hour of and preceding the flow measurements, and on the same day as the electrophysiology recordings. Brachial arterial pressure and heart frequency measurements were obtained directly after completing the flow examinations.

The examinations were performed bilaterally but the right eye was chosen as the study eye in order to allow comparisons with the electrophysiological recordings. Results were compared with age corrected normal values obtained from 84 normal subjects (ICB, unpublished data 2001).

Scanning laser Doppler flowmetry (SLDF)

Capillary flow measurements were obtained with the Heidelberg Retina flowmeter (Heidelberg Engineering, Heidelberg, Germany). The velocity, volume and flow of erythrocytes can be calculated using the Doppler effect. Several images were taken from seven areas around and on the optic disc including the optic cup at the level of lamina cribrosa. Capillary flow values were calculated by a masked investigator, using an automatic full field perfusion image analyzing program (Michelson et al. 1995 and 1998).

Color Doppler measurements (CDI)

The patients underwent CDI examinations at the Department of Clinical Physiology using an Acuson 128 instrument (Acuson Mountain View, CA, USA) with a 7.5 MHz probe. The measurements were performed according to an established technique with the patient in a supine position (Lieb et al. 1991).

The color Doppler window was localized over the retrobulbar region and the ophthalmic artery, central retinal artery and the short posterior ciliary arteries were identified. Central retinal artery (CRA) measurements were taken at the level of the optic nerve head. The ophthalmic artery was identified nasally and superiorly to the optic nerve, medially to the hypo reflective shadow representing the nerve. Measurements from the short posterior ciliary arteries were taken temporally and nasally of the optic nerve in the same plane as the CRA. A mean value from two separate measurements of peak systolic velocity (PSV) and end diastolic velocity (EDV) were calculated and the resistive index (RI) derived from the formula: RI = (PSV- EDV)/ PSV. One or two experienced technicians who were masked for patient category performed all CDI examinations.

Statistical methods

Statistical analyses of the blood flow measurements in paper II were done using the age-correlated distribution in a previously collected normal material. The mean deviation of the three measured patients was compared to a 95% prediction interval of deviation from the expected value in the normal material by a modified t-test, t= (Mean of OA population - expected mean of healthy population) / $\sqrt{}$ [(SD of residual^2 /3) + SE (of intercept in regression variable vs age)^2 - mean age of healthy population^2 x SE (of age in regression variable vs age)^2) + (mean age of OA population - mean age of healthy population)^2 x SE (of age in regression variable vs age)^2].

In paper IV the values are given as mean and 95% confidence interval. For comparison between groups the Wilcoxon´s signed rank test was used. A significance level of <0.05 was considered significant. The calculations were made in SPSS for Windows version 11.0.

Molecular genetics

Blood samples from the subjects were collected in tubes containing EDTA as anticoagulant and genomic DNA was isolated from the leukocyte fraction (Miller et al. 1988). The separated DNA was thereafter analysed at different laboratories from Sweden and abroad.

Paper I: (Department of Clinical Chemistry, University Hospital of Lund, Sweden) DNA from the patients were analyzed for alterations in *RLBP1* exon 7 by sequencing after PCR amplification (Morimura et al. 1999). The DNA sequences of the PCR products were determined on both strands by dye dideoxy sequencing using reagents in the BigDye Terminator Cycle Sequencing kit (PE Applied Biosystems). The sequences were evaluated using Sequencher (Gene Codes Corp., Ann Arbor, MI, USA).

Paper II: (Department of Molecular Genetics, Institute of Ophthalmology, University College, London and Department of Clinical Ophthalmology, Moorfields Eye Hospital, London, UK).

All 28 coding exons and splice sites of the OPA1 gene were amplified from 150ng of patient DNA and screened for DNA sequence alterations by PCR-SSCP analysis (Pesch et al. 2001; Orita et al. 1989). Each sample was then separated on an ABI 373A automated sequencer (Applied Biosystems) and the resulting DNA sequence data manually inspected for sequence alterations.

Paper V: (Department of Clinical Genetics, Sahlgrenska University Hospital, Gothenburg)

Mitochondrial DNA was examined regarding mutations known in Leber`s Hereditary Optic Neuropthy (LHON).

RESULTS

Electrophysiological findings in two young patients with mutation in the RLBP1 gene (paper I)

Two unrelated girls, 10 and 11 years old, examined at our department were found to be homozygous for a mutation in exon 7 of the RLBP1 gene, causing the amino acid substitution Arg234Trp in cellular retinaldehyde-binding protein.

Further examination demonstrated normal visual fields, and only minor changes in the fundus. The previously reported retinal changes (retinitis punctata albescens) in other patients with Bothnia dystrophy could not be verified in the present patients.

Both patients had a moderately elevated final rod threshold and a full-field ERG demonstrating an absent rod response and a normal cone response after 40 minutes of dark adaptation. When the 11 year old girl was reexamined after a prolonged dark adaptation (20-24 h), both the rod response and the dark adaptation threshold were normal. The mfERG could also be assessed in this patient, who had a normal visual acuity and a normal fundus appearance. The mfERG demonstrated a central reduction of the amplitudes in both eyes.

Full-field ERG, mfERG and mfVEP in patients with retinitis pigmentosa and residual central visual fields (Paper III)

At our department we have a registry, since 1986, of patients with heredity eye disorders. From this registry fourteen patients were selected who had been followed at the clinic for 7 to 32 years with visual acuity and visual field. They had all been examined regarding the genotype. Three patients had the RP2 genotype (linkage RP2) and two patients had the RP3 genotype (RPGRintron13splicing defect). The remaining patients are still under genetic investigation.

The ophthalmological reexamination included full-field ERG, mfERG and mfVEP.

Visual acuity varied among the patients but was at least 0.1 and most frequently better than 0.5. The visual field defect had progressed slowly in most of the patients. In one patient followed for 32 years, only minor changes of the residual visual field were seen.

In all patients fundus examination demonstrated typical changes for retinitis pigmentosa and a preserved macula without any signs of macular edema.

In twelve of the fourteen examined patients residual responses could be assessed using digital narrow band pass filters and 30 Hz flicker stimulation in the full-field ERG.
MfERG averages were compared with 10 normal individuals. In five of the patients, significant responses could be detected from the innermost region verifying a preserved central retinal function.

In the mfVEP recordings, six responses in a defined central region (sector C) where we normally detect the highest amplitudes (fig. 3) (Gränse et al. 2003), were measured and compared with normal controls. A remaining cortical response was detected in eleven of fourteen patients, representing a preserved function within 5-10 degrees of the visual field. This result validates that the mfERG signals were actually conducted through the optic pathways to the visual cortex.

Alteration of the mfVEP in a family with dominant optic nerve atrophy and in a patient during the acute phase of Leber`s Hereditary Optic Neuropthy LHON (Paper II+V)

Seven family members with the micro-deletion (1756-1767del(12bp)) in exon 18 of the *OPA1* gene were examined. The clinical characteristics varied considerably in the family. All patients had problems with glare. The visual acuity varied from 0.1 to 0.9. Color vision was also variably affected, from being normal in some patients to being severely affected in others. Fundus examinations revealed different degrees of fundus changes, from a normal appearance in some of the patients to a slight pallor of the optic disc in others. The visual fields showed central defects in all but one of the family members. The full-field ERG and the mfERG demonstrated normal retinal function in all examined patients. All patients were examined with mfVEP, which reflects the cortical visual field. The amplitudes were reduced in the above described central sector C in all of the patients, when compared to age matched healthy controls. This reduction of the amplitudes in sector C was also seen in the patient who had normal visual acuity, visual fields and color vision.
Three of the family members were examined with ocular blood flow measurements. SLDF showed a significantly decreased retinal and optic nerve head capillary perfusion. The retrobulbar blood flow velocities were significantly decreased in the central retinal and ophthalmic arteries.
Another hereditary optic disorder is LHON. A 27 year old woman with sudden visual loss in the right eye was presented at our department. She was diagnosed as having LHON with a

point mutation 11,778 identified in the mitochondrial DNA. During a period of twelve months she was repeatedly examined. At one of these examinations we found that also her left eye was diseased and therefore we were able to follow this eye during the acute phase of the disease. Initially the mean amplitude of sector C in the mfVEP response was normal in the left eye but markedly reduced in the right eye. When the left eye was affected the mean amplitude of this region of the left eye gradually decreased demonstrating a good correlation with the progression of the disease during this acute period of LHON. This pathology was correlated to the decreased visual acuity, the abnormalities in pupillary function and the visual field defects.

Interpretation of the cortical responses from the visual pathway examined with multifocal visual evoked potential (mfVEP) (paper IV)

22 normal subjects were examined with mfVEP for interpretation of the normal cortical response. Laterally placed electrodes were added to the midline electrode position (fig. 5) and a two channel registration was used as described. The right and left laterally placed electrodes were used during the same registration to assess responses from both visual cortices during the same stimulation.

The two first components in the mfVEP response were summarized and analyzed (Betsuin et al. 2001) (fig. 3). Eight regions with the highest signal-to-noise ratio (SNR) were further analyzed. Four of the regions corresponded to the nasal visual field and four of the regions corresponded to the temporal visual field (fig. 6).

When stimulating the right eye the responses from the right visual cortex revealed significantly higher amplitudes in the lower regions corresponding to the nasal visual field compared to the regions corresponding to the temporal visual field. In the responses from the left cortex there were instead significantly higher amplitudes in the lower regions corresponding to the temporal visual field compared to the regions corresponding to the nasal visual field (fig. 6).

The same significant difference was detected when stimulating the left eye. This result demonstrates that the mfVEP responses correspond to the visual pathway in which the nasal optic fibres cross over to the contralateral visual cortex (fig 6).

The same analyses of the upper field with lower SNRs did not demonstrate a similar correlation.

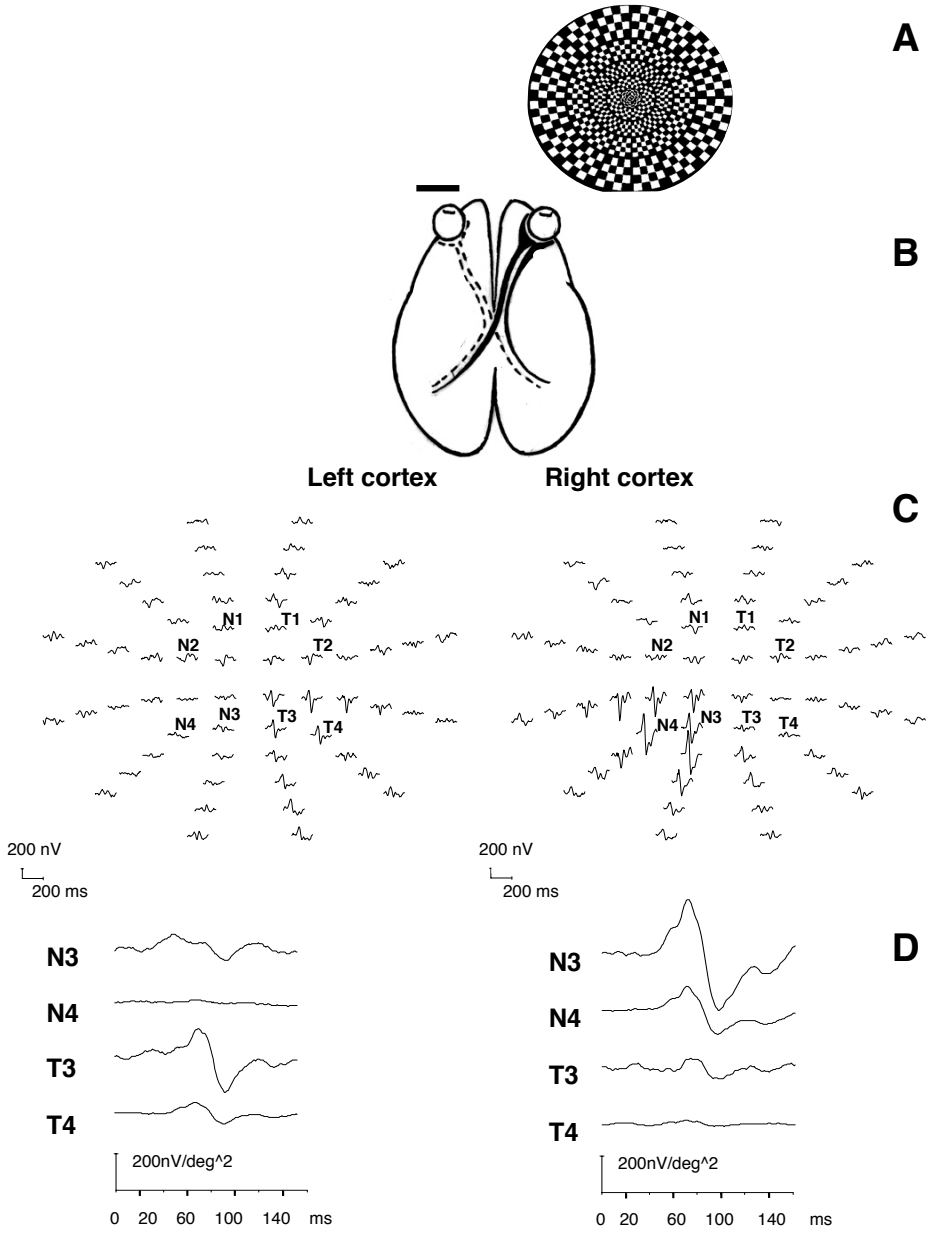

Figure 6. *An overview of the registrations from the right eye with the laterally placed electrodes. (A) The stimulation picture. (B) Schematic picture of the visual pathway of the right eye. (C) The traces from the left visual cortex and the right visual cortex. N1, N2, N3, N4 (corresponding to the regions in the nasal visual field) and T1, T2, T3, T4 (corresponding to the regions in the temporal visual field) are marked. (D) The amplitudes from N3, N4 respectively T3, T4 corresponding with the visual pathway.*

For comparison, a patient with ocular albinism was examined, as patients with this disorder are known to have abnormal visual pathways (Creel et al. 1978; Pott et al. 2003). When examining the patient's right eye the amplitudes were higher in the regions corresponding to both the nasal and the temporal visual field from the left visual cortex compared with the same amplitudes from the right visual cortex. This result proved a high ratio of crossed visual pathway.

The highest signal to noise levels measured were recorded when the laterally electrodes were used. An important finding was that these regions demonstrated significantly higher amplitudes from the right cortex compared to the left cortex.

With only the mid line electrodes, this inter-ocular difference would not be detected, nor would a further identification of the uncrossed and crossed visual pathways be possible.

DISCUSSION AND CONCLUSIONS

Electrophysiological findings in two young patients with mutation in the RLBP1 gene (paper I)

In northern Sweden, a specific form of retinitis pigmentosa has been identified and given the name Bothnia dystrophy. This autosomal recessive retinal degeneration is caused by the Arg234Trp mutation in both alleles of the RLBP1 gene encoding a cellular retinaldehyde-binding protein. Other genotypes have been found also in USA and Canada (Morimura et al. 1999; Eichers et al. 2002). In the present study we have examined two young unrelated girls from northern Sweden, both carrying the same mutation in the RLBP1 gene (Arg234Trp) in homozygous form. They demonstrated similar clinical features as described previously for Bothnia dystrophy, except for the fundus appearance that showed only slight changes in both patients, with no sign of retinitis punctata albescens.

The full-field ERG demonstrated a complete absence of the rod response after 40 minutes of dark adaptation, while the isolated cone response was normal in amplitude. After prolonged dark adaptation (20-24 h) in one of the girls the full field ERG demonstrated normal rod responses to dim blue light stimulation. It seems that the rods, when protected long enough from light, recover in patients with this disorder. Burstedt et al. also verified these findings of a gradual increase in retinal sensitivity to light and an improvement of the full-field ERG rod response with prolonged dark adaptation, especially in younger patients (Burstedt et al. 2003).

No previous reports of Bothnia dystrophy have included results from mfERG examinations. In one of the patients the mfERG demonstrated a central reduction of the amplitudes in both eyes, in spite of her normal visual acuity and normal fundus appearance.

Bothnia dystrophy in this young patient is associated with a rather unusual electro-physiological phenotype compared to other hereditary retinal disorders. The patients complaints including night blindness, glare and problems with reading correlate well to these findings.

Full field ERG, multifocal ERG and multifocal VEP in patients with retinitis pigmentosa and residual central visual fields Paper (III)

Retinitis pigmentosa is a progressive retinal degeneration characterized by a severe loss of visual function, initially affecting night vision and peripheral visual fields. The visual outcome varies markedly between different genetic subtypes, but also between different

family members from the same family. Characterization and understanding of the visual loss is important for monitoring patients with retinitis pigmentosa and for predicting visual outcome, therefore different methods have been developed for this purpose (Berson 1993).
As the retinal function in these patients often is reduced with more than 90%, it can be difficult to obtain the residual retinal responses. The standardized full-field ERG, which reflects the total retinal response, in combination with computer averaging and the use of analogue- or digital filters, have made it possible to measure, in most patients with retinitis pigmentosa, the small residual retinal response (Andréasson et. al 1988). However, in these specific patients with only small residual visual fields, the ERG may not be sensitive enough to detect the minimal regional response from the central retina. In the present study we have demonstrated that mfERG may be another objective method for measuring residual function in retinitis pigmentosa patients with small residual function in the macular region.

The mfERG can be combined with the mfVEP, another objective method used for measuring the cortical response from the central part of retina. In the present study of patients with retinitis pigmentosa with remaining small visual fields, the cortical responses measured by mfVEP demonstrate a similar preservation of central amplitudes.
In summary, this study demonstrates two new electrophysiological methods, mfERG and mfVEP, that could bee of clinical importance for evaluating and monitoring the residual central retinal function and small remaining central visual fields in patients with retinitis pigmentosa. The results also demonstrate that some patients with an atypical disease course may retain their central visual fields for many years, up to four decades.

Alteration of the multifocal VEP in a family with dominant optic nerve atrophy and in a patient during the acute phase of Leber`s Hereditary Optic Neuropthy LHON (Paper II+V)

Optic atrophy that can be inherited as a dominant disorder was first described by Kjer in 1959 (Kjer 1959). Recently the disease causing gene OPA1 has been identified (Thiselton et al. 2001). The functional mechanism underlying the OPA1 mutation segregating is unknown, but as the mutation affects the region of the protein just downstream of the conserved GTP-binding domain, it may well influence the GTPase activity of OPA1 protein.
Previous studies have revealed that affected individuals may have different degrees of visual dysfunction affecting the visual acuity, the visual fields and in some patients a reduced pattern VEP (Brown et al. 1997; Votruba et al. 1998; Johnston et al. 1999). The family in the present

study demonstrated also a considerable variability in visual function, one family member having almost no visual disturbance, normal visual fields and normal color vision. However, the result of the mfVEP was abnormal also in this patient, which stresses the importance of objective methods such as the mfVEP. Visual field testing and color vision testing have been suggested to be sensitive methods for the detection of optic atrophy (Brown et al. 1997, Votruba et al. 1998), but results from the present study demonstrates that the mfVEP which reflects the cortical activity in different parts of the visual field, may be a more sensitive and a more objective method for the detection of early optic nerve dysfunction.

Using Color Doppler and Scanning laser Doppler flowmetry, we have also documented a reduced ocular blood flow in the retina and optic nerve.

These new objective methods are valuable for assessment of the diagnosis of this particular disorder. Also they may contribute to a further understanding of the pathogenesis in DOA, which includes a change of the GTPase activity, a decreased capillary blood flow and a secondary visual disturbance in the optic pathway, confirmed by mfVEP.

We studied also another inherited optic nerve disorder which demonstrates histopathological similarities with DOA (Kjer et al. 1983). Leber`s hereditary optic neuropathy (LHON) usually has a more acute disease course (Howell 1997), but a similar reduction in the first component in the paracentral area in the mfVEP centrally was identified, as in DOA.

The mfVEP may be a valuable objective method for identifing and monitoring the disease course regarding visual loss in optic nerve disorders such as DOA and LHON.

Interpretation of the cortical responses from the visual pathway examined with multifocal visual evoked potential (mfVEP) (Paper IV)

The mfVEP is a relatively new method that objectively reflects the cortical responses from the central visual field (Baseler et al. 1994). This method offers both a topographic information from the central visual field but also the possibility to evaluate localized responses (Bengtsson et al. 2005).

Several improvements of the method have been made in order to increase its clinical value. By integrating the stimulation pattern into the IR camera it has become easier to adjust the setting to the patient. Since the IR camera is mounted on a flexible arm, the patient can lay back in a comfortable position resting his head which reduces the patients movement and thereby reduces the muscle disturbances. This is a further development of the settings for the mfVEP examination that has not been previously evaluated and described. A major advantage

of our method improvement is the possibility to continuously control the eye movements through the camera, which is especially valuable when examining patients who have problems with fixation.

Because of the anatomy of the visual cortex and the variable polarity in the spreadsheet from the mfVEP, further evaluations of these local responses are needed before interpretation of the cortical activity. The importance of responses with high SNR for the quality of the registration has previously been investigated for classic VEP recordings (Yin et al. 2004) and recently also described for the quality of the mfVEP registration (Zhang et al. 2002; Hood et al. 2003). We have demonstrated that one paracentral sector (sector C) in the mfVEP, seems to have elevated and reproducible amplitudes with high SNR (Gränse et al. 2003). This sector C, which is anatomically close to the central visual cortex and therefore of special interest, seems to be suitable for comparison between individuals.

In the present study the sectors with components with high SNR were identified in the central regions of the mfVEP traces that correspond to the crossed and uncrossed visual pathways during laterally placed electrode registration.

In the interpretation of the mfVEP these sectors with high SNR, corresponding to the lower visual field, were evaluated for identifying the pathology in the visual pathway, as in chiasmal misrouting. This was verified in a patient with ocular albinism as these patients are known to have an anomal visual pathway anatomy with several fibres from the temporal retina projecting to the contralateral visual cortex (Creel et al. 1978; Pott et al. 2003).

This further development with laterally placed electrodes and evaluation of the central areas have given us the opportunity to further compare the responses from the right and left visual cortex. These findings demonstrated that there is a significant difference in the responses with the highest SNR from the right cortex compared to the left cortex including both the crossed and uncrossed pathway. This has not previously been described and may be of major importance for better understanding and analyzing the cortical response in the mfVEP. However further research is needed to investigate if the difference between right and left cortex depends on anatomic variations or on a binocular rivalry, as previously described (Brown et al. 1997).

In summary we believe that the use of an IR camera, both for stimulation and control of fixation, is a clinically valuable improvement of the examination routine. The present study demonstrates the value of assessing doubled registrations evoked by the same stimuli by using two registration channels, which makes it possible to evaluate the visual pathway in different

disorders more specifically. In addition the study demonstrates the difference in the cortical responses from the left and right hemisphere, which may be of major importance in evaluating the recordings from the mfVEP.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Ögats näthinna är genomskinlig och är uppbyggd av flera cellager, som ligger an mot pigmentepitelet. De ljuskänsliga fotocellerna, tapparna och stavarna förmedlar en ljusstimulering via cell-lagren till ganglioncellerna som fotleder signalerna via synnerven till synnervskorsningen. Nervtrådarna från den nasala näthinnan korsar där över till andra hjärnhalvan och fortsätter tillsammans med de okorsade nervtrådarna från det andra ögats temporala näthinna i tractus opticus till laterala knäkroppen. Vid knäkroppen sker en överkoppling till synstrålen som för signalerna vidare till primära syncortex som är placerad i bakre delen av hjärnhemisfärerna.

Generna i arvsmassan kodar för olika protein som är viktiga för funktionen av näthinnan och synbanorna. Ett fel i en eller flera gener kan ge defekta proteiner som kan leda till olika skador av funktionen i näthinnan samt synbanorna. Vid ärftliga ögonsjukdomar är flera hundra sjukdoms orsakande förändringar i gener identifierade, men fortfarande är det många som är oidentifierade. Det kliniska uttrycket för en förändring i en gen (genotyp) kallas fenotyp. För att identifiera gendefekten och därmed förstå sjukdomsprocessen (patogenesen) är det av största vikt att beskriva och klargöra fenotypen så objektivt och exakt som möjligt. Ett viktigt hjälpmedel till de traditionella kliniska undersökningarna för att objektivt studera funktionsstörning av näthinnan och synbanorna är elektrofysiologiska undersökningar.

Elektroretinografi (ERG) mäter via en kontaktlins på ögat de elektriska signaler som uppstår då näthinnan träffas av ljus. Genom att stimulera med ljus av olika våglängd samt varierande ljusstyrka kan man separera funktionsstörningen från tapparna respektive stavarna.

Även om näthinnefunktionen är reducerad med mer än 95 %, så kan patienten ha viktig kvarvarande synfunktion, men det ställer då större krav på våra metoder att kunna registrera denna synreduktion. Utveckling av ERG med olika filter och förstärkningsteknik har gjort det möjligt att även mäta mycket små signaler.

Senaste åren har en ny teknik, multifokalt ERG (mfERG) utvecklats. Där används en multifokal stimulerings teknik som gör det möjligt att mäta funktionen från mycket små delar av den centrala näthinnan, gula fläcken. Gula fläcken stimuleras med en mönsterbild uppdelad i vanligtvis 103 små stimuleringsområden. Svaren från dessa avleds även här via en kontakt lins på ögat.

Vid multifokalt VEP (mfVEP) används samma multifokala stimulerings teknik men svaren registreras via elektroder placerade i nacken. Svaren avleds därmed från synbarken istället och man kan då klarlägga att signalerna från näthinnan via synbanorna når fram till synbarken.

Stimuleringsbilden består här av 60 st. sektorer av varierande storlek och utseende innehållande ett shackrutmönster. Man får ett svar från synbarken innehållande 60 st svarsamplituder som motsvarar det centrala synfältet (ca 20-25˚ från fovea).

De olika elektrofysiologiska undersökningarna, kan tillsammans kartlägga funktionsstörningen. Om t.ex. ERG och mfERG visar på normala värden men mfVEP är reducerat kan man misstänka skada på synbanorna.

I det här arbetet är beskrivet olika ärftliga ögon sjukdomar där utvecklingen av elektrofysilogin och modifiering av undersökningarna visat stort värde.

I arbete I undersöktes två flickor som hade en känd mutation i RLBP1 genen. Denna typ av retinitis pigmentosa (rp) som kallas Bothnia dystrofi har en något annorlunda klinisk bild. Tidigt får patienterna påverkat mörkerseende (stavfunktionen) utan påverkan på tappfunktionen. Längre fram får patienten en skada även på den centrala synfunktionen. De båda flickorna uppvisade i princip helt normala ögonbottnar och inte de klassiskt beskrivna vita fläckarna i ögonbotten som man vanligen associerar med denna typ av retinitis pigmentosa. ERG visade kraftigt reducerat stav svar och i princip normalt tapp svar hos båda flickorna.

Den ena flickan med normal synskärpa och synfält undersöktes vid två tillfällen. Vid andra undersökningstillfället förlängdes mörkeradaptaionstiden till 24 timmar, varvid man fann normal stavfunktion. Undersökningarna visade att när stavarna skyddas tillräckligt länge för ljus, så kan de återhämta sig åtminstone i det tidiga skedet av Bothnia dystrofi. Den här patienten undersöktes också med mfERG som visade klar funktions nedsättning i gula fläcken vilket verifierar hennes subjektiva synbesvär. Med modern elektrofyisologisk apparatur, mfERG och ERG, fann vi således att man tidigt kan följa påverkan av den centrala funktionsstörningen trots att ögonbottenfynden och syn funktionen kan te sig normal.

Retinitis pigmentosa är en sjukdom i ögat som karaktäriseras av svår synnedsättning pga en successiv förtvining av näthinnan. Tidigt i sjukdomsförloppet är patienten ofta bländningskänslig, har dåligt mörkerseende och utvecklar långsamt tilltagande defekter i synfältet. Tidigare studier har visat att patienter med retinitis pigmentosa i genomsnitt förlorar ca 16 % av synfunktionen/år. Dock har det visat sig att ca 20 % av retinitis pigmentosa patienterna inte följer denna typiska progress av sjukdomen utan kan ha bevarad central synfunktion av olika grad under många år, tom under hela livet. I arbete III undersökte vi 14 patienter med retinitis pigmentosa, med bevarad central synfunktion, som hade följts vid vår specialmottagning för utredning av ärftliga näthinnesjukdomar under minst 7 år. Med hjälp av

ERG, mfERG och mfVEP gjorde vi en uppföljande undersökning av de 14 patienterna. MfERG och mfVEP visade stort värde för att närmare kartlägga och följa dessa patienter med långsam sjukdoms progress. Centralt svar kunde identifieras med mfERG hos flera av patienterna och hos dessa kunde mfVEP verifiera att signalerna fortleds till synbarken.

Dominant optikus atrofi är en ärftlig sjukdom i synnerven som drabbar det centrala seendet och färgseendet. Sjukdomen visar på en mycket varierande fenotyp mellan olika familjer men även inom samma familj. Patienterna besväras ofta av en uttalad bländningskänslighet och symtomen progredierar under åren. På grund av varierande grad av syn störningar och grad av progress kan det vara svårt att identifiera dessa patienter. Detta är dock viktigt, framförallt för barn så att de får rätt hjälpmedel så tidigt som möjligt, särskilt i studiesituationen.

Orsaken till sjukdomen har tidigare varit okänd, men för ett par år sedan identifierades ett protein (DOA-1). Förändringar i det proteinet har visat sig vara en vanlig orsak till sjukdomen. En stor familj med dominant optikus atrofi identifierades. Med hjälp av elektrofysiologiska undersökningar: ERG, mfERG samt mfVEP undersöktes familjen i arbete II för att klarlägga fenotypen. I samarbete med ögonkliniken i Malmö gjordes även blodflödesmätningar på synnerven hos tre av patienterna. Blodprov togs för DNA-analys och mutation på OPA-1 genen identifierades hos alla undersökta familjemedlemmar. Fullfälts ERG och mfERG visade normal näthinnefunktion hos alla patienterna. Däremot visade mfVEP central reduktion i svaren hos alla i familjen, även hos den patienten som hade normal synskärpa, synfält och färgseende. Blodflödesmätningarna visade ett reducerat flöde i synnerven och retrobulbärt. MfVEP och blodflödesmätningarna över synnerven visar således möjlighet att objektivt kunna påvisa synnervs påverkan hos dessa patienter med dominant optikus atrofi.

En annan ärftlig synnervs sjukdom som har en hastigare sjukdoms progress men med samma histopatologiska bild som dominant optikusatrofi är Leber`s hereditära optikusatrofi (LHON). En kvinna med denna sjukdom beskrivs i arbete V. Hon följdes under ett års tid vid ögonkliniken i Lund med prövning av synskärpa, synfält, pupillfunktion och mfVEP. Vid första undersökningarna var endast höger öga påverkat men efter 5 mån blev även vänster öga påverkat, vilket gjorde att man kunde följa detta öga under det akuta insjuknandet. MfVEP uppvisade samma centrala reduktion som vi såg hos familjen med dominant optikus atrofi. Den tilltagande reduktionen av amplituderna i detta område visade god korrelation med sjukdomens förlopp. Vid dominant optikus atrofi och LHON kan därför mfVEP vara en värdefull metod för att identifiera en synförlust samt följa denna under sjukdomsförloppet.

Standard VEP med stimulering med ljus eller mönster har under flera år varit grunden för undersökning av funktionen i synbanorna och synkortex. En ny undersökningsmetod för detta ändamål som har visat sig vara av stort intresse är mfVEP. Metoden har som ovan beskrivits sitt ursprung från mfERG.

Undersökningssituationen utvecklades så att stimuleringsbilden nu visades på en liten skärm i en IR-kamera nära ögat jämfört tidigare undersökningar och beskrivningar där patienten sitter framför en större fix skärm. Denna modifiering av undersökningen gjorde det lättare att anpassa undersökningen till patienten, som nu kunde ligga avslappnat tillbakalutad vilket minskade muskelstörningarna från nacken. Likaså kunde fixationen av ögat kontrolleras via kameradelen av IR-kameran.

Svarsamplituden vid mfVEP för olika delar av synfältet kan dokumenteras i form av ett spread sheet och kan beskrivas som en form av synfältsundersökning. Genom att lägga till ytterligare en registreringsavledning, kan man samtidigt avläsa svaret från både höger och vänster synkortex under samma stimulering. Med denna nya undersökningsmetod får man möjlighet att jämföra och korrelera dessa svarsamplituder och på så sätt kartlägga synbanornas funktion.

I arbete IV undersöktes 22 normala personer med mfVEP med IR-kamera och samtidig registrering från både höger och vänster synkortex. Undersökningarna visade att man kan åskådliggöra de korsade och okorsade synbanornas funktion med denna metodik. I motsats till tidigare standard VEP med två avledningar som visar totalfunktionen på höger och vänster sida, så visar mfVEP hela synfältets funktion på vardera sida. En intressant observation var också att de områden med högst svars amplituder visade signifikant högre amplituder från höger synkortex jämfört med vänster synkortex, både för de korsade och de okorsade synbanorna.

Vår förhoppning är att man i framtiden skall kunna behandla en ärftlig ögonskada när man lyckats identifiera vilket protein som är skadat. Detta kan ta mycket lång tid. Under tiden är det av största vikt att vi på ett tidigt stadium försöker identifiera den genetiska störningen hos patienterna, och framförallt att vi klarlägger synhandikappet och prognosen på ett så objektivt och klart sätt som möjligt. Det är en förutsättning för att patienten ska få lämplig hjälp och synrehabilitering vilket är särskilt viktigt i unga år. För det behövs en bra fenotypbeskrivning vilket underlättas av utvecklingen av de elektrofysiologiska undersökningsmetoderna.

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