INTRODUCTION

Night blindness is a problem commonly reported by visually impaired patients. It can present itself in several ways: young children that are scared at night, or stumble during night walks, adults that are hesitant to drive at night, or have reading problems when there is not sufficient light, etc. It is up to the rehabilitation centre to implement a strategy on subsequent interventions.

In the following sections, I will first introduce the Bartiméus institution and the International Classification of Diseases (ICD) and the International Classification of Functioning, Disability and Health (ICF). Then I will describe what night blindness is and what might be the causes of night blindness. As background, some basic knowledge is provided on the retina and electrophysiology (a diagnostic tool used in ophthalmology). Next, I will introduce the main subjects of this thesis: the complexity of the rod system which supplies us with our night vision, the retinal disorder called congenital stationary night blindness, and the measurement of scotopic visual functions (visual functions in the dark). Finally, I will summarize the aim of this study and give an outline of this thesis.

Bartiméus

The Bartiméus institute was founded in 1916 and provides rehabilitation, care, and education for visually impaired people. In the early years, most admitted patients were blind and lived at the institute. Nowadays, most patients are visually impaired and live at home, go to a local school, or work, just like anyone else. This shift reflects the ongoing changes in health care that more and more focuses on the independence of patients. Patients no longer come to Bartiméus for “help” but with a specific question. This also changed the activities of Bartiméus from “taking care of” patients towards “assisting” patients to live their own independent life. The institute, for instance, provides courses to walk with a cane, or advises on light levels at work or school to optimize a patient’s visual functions. These are practical forms of assistance. However, rehabilitation also includes consultative forms of assistance such as: clarification of the visual disorder of the patient, explaining what he or she can and cannot see, and provide a prognosis. These aspects
are especially important for young patients and their parents. Depending on the patient’s question, rehabilitation comprises different aspects and steps. Therefore, the institute uses the ICD and ICF structure.

**ICD and ICF**

The World Health Organisation (WHO) owns and publishes the international classifications on health, including the ICD, ICF. The ICD, founded in 1853, is an etiological framework for diagnostic classification. In 2002, the ICF was added to complement the ICD, in terms of the consequences of the disease in daily life. The ICF is structured around the following broad components: body functions and structure (e.g. visual acuity), activities (e.g. reading) and participation (e.g. going out at night).

The ICD and ICF provide an unified and standard language and framework for the description of diseases and health. The classifications have several applications such as the international comparison of data. They can also be used as a communication tool between professionals from different fields, for instance in making an agreement on the financial support provided by the government for visually impaired persons. Another application of the ICD and ICF is to structure rehabilitation! Patients who are referred to the rehabilitation centre usually have questions on the level of activity or participation. To properly answer these questions, all ICD and ICF components should be assessed. This subsequently comprises determination of the diagnosis, measurement of a patient’s visual functions, and an inventory of a patient’s activities and participation. This approach ensures that interventions are applied at the appropriate level. For instance when a young patient has difficulties reading the books at school, the solution depends on the diagnoses, the patient’s visual functions and his environment. When the diagnosis is a progressive disorder, a solution could be to learn braille. However, in case of a stationary disorder and fairly good visual acuity, a solution could be to use enlarged versions of the books or a laptop. But sometimes, the solution can be even more simple. When the patient is photophobic (intolerant to light) but has a good stationary visual acuity, changing his seat from the window to a darker position in the room could be sufficient. Thus, only after the patient’s situation is charted fully, the rehabilitation centre can inform, advise and assist
the patient on his or her problem in appropriately.

Night blindness
Night blindness is a complex symptom that may have several causes. In adults, impaired night vision is usually caused by ageing of the lens (Figure 1.1) which makes it more cloudy. When we focus on an object with a cloudy lens, part of the reflections from the object are scattered over the retina. We call this stray light, or glare. Glare is especially problematic in the dark when there is a light source in the field of vision, for instance the headlights of an approaching car. In severe cases, glare can make it impossible to see anything but the scattered light from the light source. Although glare may lead to problems in the dark, it is not really night blindness. Glare leads to problems with contrasts, while night blindness leads to problems when it is dark. There are several retinal disorders that can cause night blindness, of which the most important are retinitis pigmentosa (RP) and congenital stationary night blindness (CSNB). In these retinal disorders, the rod system does not function correctly. In both disorders, the electroretinogram (ERG) is essential for diagnosis.

The retina
The human eye contains four types of photoreceptors that contribute to our vision: the rods and three types of cones. During the day, the cone system supplies our brain with signals so that we can see the world sharp and colourful. But when it is dark and the rod system takes over, our visual acuity is decreased (we see less details) and we cannot see colours. The rod system comprises about 60 million rods (compared to only ca. 3 million cones) and each rod can respond to a single photon. It is due to this system that the humans can still see at starlight conditions when there are only few photons per second entering the eye. On the other hand, cones generate signals even when the retina is stimulated by a shower of photons emerging from bright sunlight. Due to this dual system, humans are able to see remarkably well over a range in which illumination changes by more than a factor of $10^{11}$, or 100-billion.

Besides the different sensitivity of rods and cones, also the way retinal signals are
processed contributes to the efficiency of the visual system. The signals from the photoreceptors are transferred through four different cell layers before reaching the brain (Figure 1.1). The rod system comprises the rods and the pathways that transfer the rod signals. The same applies for the cone system. In the most direct pathway, the signals from the photoreceptors travel via bipolar cells to the ganglion cells. The axons of the ganglion cells form the optic nerve that enters the brain. The horizontal and amacrine cell layers form lateral connections in the retina so that signals from one part of the retina influence signals in another part of the retina. The human retina contains about 10 types of bipolar cells, up to 10 types of ganglion cells, three different types of horizontal cells, and up to 20 types of Amacrine cells. This complex network results in highly specialized signal processing. Examples of this signal processing are contrast and brightness adjustments, which improve our daytime or night time vision, or detection of movement in a certain direction.

The electroretinogram
In electroretinography (ERG), the summed activity of the cells in the retina is recorded while the eye is stimulated by flashes of light. The stimuli are generated by a homogeneously

Figure 1.1 A schematic presentation of the eye and the retina. (Figure derived from: http://webvision.med.utah.edu, and published with permission.)
illuminated bowl: the Ganzfeld stimulator. In our department at Bartiméus, ERGs are performed without general anaesthesia or sedation. The retinal activity is recorded through DTL electrodes that float on the cornea, after the cornea is anaesthetized with eyedrops. We use the DTL electrodes in almost all patients, mostly children. Only in infants we use contact lens electrodes. To eliminate the influence of the pupil diameter on the amount of light, the patient’s pupils are dilated. ERGs are recorded after the patient is either dark adapted for 20 minutes or light adapted for 10 minutes. Depending on the stimulus, the activity of different retinal cells can be recorded, especially the cells in the direct pathway: rods, cones, and bipolar cells. The amplitude of the ERG is in the order of microvolts.

The International Society for Clinical Electrophysiology of Vision (ISCEV) recommends a protocol that (minimally) includes four ERG measurements:

1. The dark adapted 0.01 cd·s·m⁻² ERG, also called “rod ERG” or “scotopic ERG”.
2. The dark adapted 3.0 cd·s·m⁻² ERG, also called “rod-cone ERG” or “mixed ERG”.
3. The light adapted 3.0 cd·s·m⁻² ERG, also called “cone ERG” or “photopic ERG”.
4. The light adapted 3.0 cd·s·m⁻² 30 Hz flicker ERG.

These ERGs are plotted in Figure 1.2 from top to bottom. During the recording of the DA 0.01 ERG, the stimulus is so dim that only rods are stimulated. This ERG consists of a positive “b-wave”, caused by depolarization of bipolar cells. The signals from the rods themselves are too small to be discriminated on the ERG. The stimulus used to record the DA 3.0 ERG is much brighter so that rods and cones are activated. Hyperpolarization of the photoreceptors causes a negative “a-wave” on the ERG. The LA 3.0 ERG is measured on a background intensity that desensitizes the rod system. This ERG shows mainly cone activity. Activity from the cone system is completely isolated when a high frequent stimulus (30 Hz ERG) is used, as the rod system is not able to react to high flickering stimuli. At our department, we normally use an extended ISCEV protocol that includes additional stimulus intensities in the dark adapted and light adapted ERGs. The advantage of the extended series is that it improves the evaluation of the ERGs. However, these extended measurements are not directly used in this thesis.
Primary and secondary rod pathway

The human retina contains about 20 times more rods than cones. However, it is thought that in evolution the cone system was first evolved and formed a pre-existing framework of cells in the retina, upon which the rod circuitry was superimposed. While cone signals travel through the retina by a direct pathway (photoreceptor → bipolar cell → ganglion cell), rod signals first need to step up on the cone pathway. Today, at least three rod pathways have been distinguished, called the primary, secondary and tertiary rod pathway. They differ in sensitivity, the primary being the most sensitive. The function of the tertiary rod pathway and its relevance for human vision is still unclear, and will not be discussed in this thesis.

The primary rod pathway is visualized in Figure 1.3 as ON1 and OFF1. In this pathway,
rod signals are first transferred to rod ON bipolar cells through a distinctive synaptic mechanism able to transmit single photon signals. Then, the signals travel via All amacrine cells and cone ON bipolar cells to ON ganglion cells (ON1) or via cone OFF bipolar cells to OFF ganglion cells (OFF1)
\[12,14,15\]. The primary rod pathway is most effective at very low intensities. Through this pathway, a single photon signal from one rod can be singled out from the noise generated by 10,000 “silent” rods\[15\]. The secondary rod pathway is less sensitive than the primary rod pathway and effective at higher intensities (ON2 and OFF2 in Figure 1.3). In this pathway, the rod signals travel via gap junctions between rod and cone pedicles to “piggyback” onto a direct cone pathway: cone → ON cone bipolar cell → ON ganglion cell (ON2) or cone → OFF cone bipolar cell → OFF ganglion cell (OFF2)\[11,16,17\].

The existence of the primary and secondary rod pathways in humans was discovered through psychophysical experiments by Conner et al.\[18,19\]. Subjects were asked to look at a flickering light stimulus and to report whether they saw the flicker or not. Conner et al. used a dim 15 Hz flicker, that was subsequently increased in intensity. Normally, when the intensity of a flickering light stimulus is increased, the perception of flicker becomes more and more conspicuous. However, for a flicker of 15 Hz, the perception of flicker
decreased at a certain intensity and then reappeared while the intensity of the stimulus linearly increased\textsuperscript{18-22}. It is thought that this phenomenon is caused by the destructive interference of signals from the two pathways. The flicker cancellation is schematically presented in Figure 1.4. When stimulated with a 15 Hz flicker, the signals from the two rod pathways roughly represent a 15 Hz sine wave. When the signals from the primary and secondary rod pathway are of equal amplitude but of opposite phase, the sum of the signals represents a continuous signal in which the 15 Hz component is no longer present.

In later studies, it was found that this “15 Hz phenomenon” could also be recorded on ERGs. When the amplitude of the 15 Hz ERG signal was plotted against the stimulus intensity, a minimum appeared. The stimulus intensity of the minimum corresponded to the intensity at which subjects reported disappearance of flicker perception\textsuperscript{23,24}. Furthermore, the 15 Hz ERGs, that roughly resembled a sine wave, turned about 180° in phase between the intensities above and below the intensity of the minimum. This observation supports the explanation of the phenomenon as presented in Figure 1.4.

On standard dark adapted ERG, the functioning of the rod system can be determined, but a distinction between the primary and secondary rod pathway is not possible. However, determining the functioning of the two rod pathways separately may be clinically valuable and contribute to our understanding on night blindness. Because the 15 Hz

\begin{figure}[h]
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\includegraphics[width=0.5\textwidth]{figure1_4.png}
\caption{Signal flicker cancellation at 15 Hz by destructive interference of signals from the primary and secondary rod pathways.}
\end{figure}
phenomenon can be objectively measured on ERGs, 15 Hz ERGs may be used to examine the functioning of the primary and secondary pathways. If no minimum is recorded in a patient, this indicates that one of the two rod pathways is not functioning. However, it is unclear to which extent and at which intensities cone signals contribute to the 15 Hz ERGs. Therefore, we first investigated the origin of the signals recorded at 15 Hz, especially the contribution of the cone pathway, to improve upon the interpretation of (abnormal) 15 Hz ERGs measured in patients. Next, we designed a clinical protocol which we applied in 20 healthy subjects to determine normal ranges, and in patients to investigate the usefulness of a clinical application.

**Congenital Stationary Night Blindness (CSNB)**

Besides retinitis pigmentosa, congenital stationary night blindness (CSNB) is the main retinal disorder that may cause night blindness. The Schubert-Bornschein type of CSNB is characterized by an “electronegative” dark-adapted 4.0 ERG (black line in Figure 1.5). The normal a-wave but the decreased b-wave reflect the cause of disorder: a signal transmission defect between the photoreceptors and the bipolar cells. CSNB can be subdivided into CSNB1, also called “complete CSNB”, and CSNB2, also called “incomplete” CSNB. The two types can be distinguished on ERGs: CSNB2 patients show reduced but recordable rod function and reduced cone function, CSNB1 patients have no residual rod function but near normal cone function.

CSNB is a hereditary disorder, caused by DNA mutations. The human DNA is organized into 46 chromosomes. Two of these are sex chromosomes: two X chromosomes in females and an X and Y chromosome in males. The other 22 chromosome pairs are called autosomes. One part of a pair of chromosomes is inherited from the father, the other one from the mother. Each gene on the chromosomes encodes for a protein with a specific function in the human body. When a gene code contains an error or mutation, the protein will be missing or dysfunctional. When a mutation on both gene copies is required to cause a disease, the mode of inheritance is called autosomal recessive. When the genetic mutation occurs on the X-chromosome, men do not have a chromosomal counterpart. Therefore, in the X-linked inheritance form predominantly males are affected, while
women are typically only carriers of the mutated gene.

CSNB segregates in both the X-linked (xl) and autosomal-recessive (ar) form. Since 1999, six genes have been identified that cause CSNB with electronegative ERG. To date, four genes are associated with CSNB1: *NYX* (xl)\textsuperscript{7,29}, *GRM6* (ar)\textsuperscript{28,30}, *TRPM1* (ar)\textsuperscript{31-33} and *GPR179* (ar)\textsuperscript{34}. The *TRPM1* gene has been identified in collaboration between Bartiméus and the Netherlands Institute for Neuroscience during this PhD project (Chapter 4). Two disease genes have been implicated in CSNB2: *CACNA1F* (xl)\textsuperscript{35}, and *CABP4* (ar)\textsuperscript{36-37}. Figure 1.6 shows the location and function of the proteins that are encoded by the CSNB genes. Mutations in CSNB1 genes affect the function of proteins that are localized on the dendrites of the depolarizing or ON bipolar cell\textsuperscript{37}. Because rod signals primarily travel through ON bipolar cells, this could account for a non-functional rod system\textsuperscript{36}. CSNB2 genes encode for proteins that are localized at the synaptic terminal of photoreceptors. *CACNA1F* and *CABP4* are involved in continuous calcium-dependent neurotransmitter release. In CSNB2 patients, the signal transmission from both rods and cones is impaired\textsuperscript{38}.

Due to the signal transmission defect, CSNB patients show a variety of visual abnormalities. Symptoms associated with CSNB are: high refractive errors, nystagmus (involuntary eye movements), reduced visual acuity, and night blindness. Because CSNB is a rare retinal disorder, much is still unclear regarding the symptoms and their variability. Bartiméus has a relatively large cohort of CSNB patients. In this large group of patients we investigated:

\textbf{Figure 1.5} A representative example of the dark adapted 4.0 ERG of a patient with CSNB (black) and a normal ERG (grey). The ERG reflects the defective signal transmission between photoreceptors and bipolar cells that causes CSNB: the a-wave is approximately normal, but the b-wave is decreased.
Figure 1.6 Schematic representation of the location and function of the proteins that are encoded by the CSNB genes. Mutations in CACNA1F and CABP4 can cause CSNB2. The CSNB2 "proteins" are localized at the photoreceptor side and are involved in continuous calcium-dependent neurotransmitter (glutamate) release. Mutations in NYX, GRM6, TRPM1 and GPR179 can cause CSNB1. Nycalopin is the protein encoded by NYX, and mGluR6 is encoded by GRM6. The CSNB1 "proteins" are required for reception of glutamate and further signal transmission through the bipolar cell.

(1) the relative frequency of the genetic causes of CSNB1 and CSNB2, and (2) genotype-phenotype correlations in CSNB1 and CSNB2. To date, the 101 patients included in our study (Chapter 5) represent the largest number of CNSB patients in which phenotype-genotype correlations were investigated.

Measuring scotopic visual functions in patients

For adequate rehabilitation, it is important to be able to determine a patient’s visual functions at dark or scotopic circumstances. Knowing a patient’s scotopic visual functions makes it possible to predict a patient’s restrictions and to advise on aids. This is especially important for parents of young patients. At present, the dark adaptation (DA) curve is the only widely used psychophysical scotopic functional tool, (see Figure 1.7). Before the DA curve can be recorded, the patient is first light adapted for 5 to 10 minutes. Next, the room is completely darkened and the patient is asked to indicate whether he or she can see a dim light stimulus. The intensity of the stimulus is controlled by the examiner. During
Figure 1.7 A representative example of a normal dark adaptation (DA) curve. The threshold is plotted on a logarithmic scale. The DA curve is recorded after a period of light adaptation (not in figure). During 30 minutes of dark adaptation, the threshold for the dimmest perceivable light stimulus decreases as the sensitivity of the photoreceptors in the retina increases. As the cone system more quickly adapts to the dark than the rod system, the first five minutes of the curve are determined by the cone system. After five minutes, the rod system becomes the most sensitive system and determines the curve.

The recording, which usually takes 20 to 25 minutes, the examiner repeatedly determines the dimmest stimulus the patient can see. During the measurement, the rods and cones adapt to the dark and a more and more dim stimulus can be perceived. At the end of the recording, the final elevation of the DA curve is determined by comparing the intensity of the dimmest light stimulus perceived by the patient with that of normal subjects.

In the Netherlands, driving a car at night is legally restricted if the DA threshold is more than 1 log elevated. However, the DA curve provides only limited aspects of a patient’s scotopic visual functions. For instance, it does not measure scotopic visual field restrictions. Furthermore, it does not measure the scotopic functions of a patient at light intensities that are representative for an illuminated street in the western world. From our lab experience, we knew that the elevation of the DA curve did not always correspond to the patient’s experience of night vision problems. We therefore needed more and different measurements to evaluate a patient’s scotopic functions.

In the final part of this thesis, we assessed night vision problems in CSNB patients. Because
CSNB patients have impaired transmission of rod signals, their DA curve is always elevated. However, night vision problems in CSNB patients have never been investigated in a systematic way. Better insight into these problems may result in better rehabilitation and better information for patients and their parents. We therefore invited young CSNB adults and asked them to participate in the study. We developed a questionnaire on night vision problems and used three scotopic functional tests for objective measures. With these tests we determined absolute thresholds, scotopic visual fields, and recorded the detection and recognition of objects at intensities that ranged from very dark to bright intensities.
SUMMARY OF THE AIM AND OUTLINE OF THE THESIS

Professionals at Bartiméus use the ICD and ICF as a framework to investigate the functions and participation problems a patient may have. However, when it comes to night blindness, much is unclear on both the ICD and ICF domains. Measurements of a patient’s scotopic visual functions, the correlation between scotopic visual function’s and disabilities at night, and evidence based advice or aids, are ICF areas for which there is little available. Furthermore, much is still unknown about the retinal structure and functions of the rod system, and the genetic origin of the retinal structure. This thesis comprised research on aspects of night blindness and night vision in both the ICD and ICF domain.

Firstly, we investigated whether it is possible to examine the functioning of the primary and secondary rod pathways in patients through dark adapted 15 Hz ERGs. In Chapter 2, we investigated the origin of the ERG signals, and especially cone pathway contributions to 15 Hz ERGs. This study resulted in a clinical 15 Hz protocol. In Chapter 3, we applied this protocol in 20 healthy subjects to determine normal ranges, and in CSNB patients and an achromat to substantiate our hypothesis on the different pathway contributions and to investigate the clinical use of the protocol.

Secondly, we investigated clinical, electrophysiological and genetic aspects of the retinal disorder CSNB. In Chapter 4 we demonstrated that mutations in the TRPM1 gene cause an autosomal recessive variant of CSNB1. Chapter 5 describes a genotype-phenotype study on 101 Dutch CSNB patients. The study included DNA mutation analysis, full ophthalmic examinations, and ERG measurements.

Finally, we performed a study on the symptom night blindness. Chapter 6 describes an assessment of night vision problems in CSNB patients. The assessment included a questionnaire and three tests that measure different aspects of the scotopic visual functions. A general discussion on the studies and thoughts on future research are given in Chapter 7.
REFERENCES


General introduction